

Hypoglycaemia in adult humans, with and without type 1 diabetes and impaired awareness

A thesis by

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Dissertation presented for the degree of
MD Doctor of Medicine

University of Edinburgh

2011

Declaration

- a) This thesis was composed by Dr Jacqueline Geddes
- b) Study 1 was performed, analysed and written primarily by myself. In study 2 Dr Rohana Wright and I executed the glucose clamp procedures. The data was analysed in part by Professor Roger Ratcliff, Ohio State University. Study 3 was performed, analysed and written primarily by myself. Dr Rohana Wright assisted in the data collection. In study 4 the original study was performed by Dr NN Zammitt. The data was gathered and analysed by myself. The paper was co-written with Dr Pratik Choudhary. Study five was performed, analysed and written by myself.
- c) I hold the degree MB ChB
- d) This thesis has not been submitted for any other degree, diploma or professional qualification.

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ABSTRACT OF THESIS

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<i>Degree</i>	MD Doctor of Medicine
<i>Title</i>	Hypoglycaemia in adult humans, with and without type 1 diabetes and impaired awareness.
<i>Word Count</i>	Approx (main text) 28,000

Hypoglycaemia is a very common side-effect of insulin therapy for diabetes and directly affects cognitive function. It can be identified by the onset of symptoms and by blood glucose monitoring. Impaired awareness of hypoglycaemia is an acquired syndrome in people with insulin-treated diabetes. The definitions, frequency, causes, treatment and prevention of clinical hypoglycaemia and the effects on, and moderators of, cognitive function will be discussed.

Two studies have examined the effects of hypoglycaemia on tests of particular cognitive domains in subjects with and without type 1 diabetes. Three further studies have examined the frequency of hypoglycaemia in people with and without impaired awareness, the prevalence of impaired awareness of hypoglycaemia (IAH) and have compared methods of assessing awareness of hypoglycaemia.

In study 1 the effect of acute hypoglycaemia on psychomotor function was examined in healthy volunteers ($n=20$) and adults with type 1 diabetes ($n=16$). Although acute hypoglycaemia caused significant impairment of several psychomotor functions in non-diabetic adults, a lower magnitude of impairment was observed in those with type 1 diabetes. The potential mechanisms behind this are discussed.

In study 2 the effect of acute hypoglycaemia on a simple two-choice reaction time test, which has a model with validated performance parameters, was examined in 14 non-diabetic volunteers. Application of the validated model to the results of this task revealed that hypoglycaemia affected central processing and was not related to the amount of evidence required to make a decision or to peripheral and motor processes. This study is the first to use this method to dissect the effects of hypoglycaemia on cognition and enhances understanding of the mechanism underlying neuroglycopenia in adults.

In Study 3 the methods of evaluating awareness of hypoglycaemia were compared in people with type 1 diabetes. Good concordance in clinical characteristics and frequency of biochemical hypoglycaemia was observed between the methods described by Gold et al and Clarke et al but not with a Danish method.

In study 4 continuous glucose monitoring (CGM) and home blood glucose monitoring were performed prospectively for 12 months in people with and without IAH. Those with IAH had a 1.6-fold higher incidence of biochemical hypoglycaemia as demonstrated by blood glucose monitoring, but CGM did not identify patients with IAH.

In study 5 the prevalence of IAH in a large clinic population with type 1 diabetes was estimated and compared with earlier assessments. The overall prevalence was 20%.

Acknowledgements

I am deeply indebted to Professor Brian Frier and Professor Ian Deary who acted as co-supervisors during my research fellowship.

Professor Frier has instilled in me a love of clinical research, which hopefully in time I will be able to instil in others. He has generously given up his time and effort, not only to me but to numerous previous research fellows, in a bid to try and improve understanding of the clinical and pathophysiological effects of hypoglycaemia.

I am extremely grateful to Professor Ian Deary, for giving me an insight into the world of cognition and cognitive function testing. He also has been very generous with his time explaining the finer points of statistical analysis to me.

I thank Nicola Zammitt and Rohana Wright, with whom I shared an office with. They were there for all the highs and the lows.

I would also like to thank all the research nurses of the clinical research facilities at the Royal Infirmary of Edinburgh and the staff and patients of the Royal Infirmary of Edinburgh who acted as willing participants in my research studies.

Dedication

For Kenneth, Eilidh and Angus.

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Chapter 1

Pathophysiological and Clinical Aspects of Hypoglycaemia

1.1 Introduction

Hypoglycaemia remains a common and feared side effect of treatment with insulin [1,2]. In addition to affecting most people with insulin-treated diabetes, it is also associated with sulphonylurea therapy in people with type 2 diabetes, but occurs much less frequently [3].

In people without diabetes physiological insulin secretion is minimal in the fasting state, however once the blood glucose concentration starts to rise, insulin concentrations increase almost ten-fold within three to five minutes of the glucose rising. Over time this lowers blood glucose concentrations and insulin secretion returns to basal levels. For people with insulin-treated diabetes treatment should ideally aim to provide a method of insulin delivery that replicates normal physiology. Despite improvements in insulin therapies, intensification of insulin regimens and innovative patient education the current methods of insulin replacement therapy for people with diabetes are far from physiological and marked variations occurring in intra- and inter-individual absorption of insulin. Thus hypoglycaemia remains almost an inevitable consequence of insulin therapy.

Hypoglycaemia *per se* generates a range of unpleasant and uncomfortable symptoms that requires the person with diabetes to treat themselves to correct the condition. If untreated or if the symptoms have been lost, it can lead to confusion or collapse, creating a significant risk of morbidity and mortality. Given this, in people with insulin-treated diabetes, it is not surprising that fear of hypoglycaemia can lead to barriers in attaining good glycaemic control [2].

Good glycaemic control however is necessary to prevent diabetes-related complications. In people with type 1 diabetes The Diabetes Control and Complications Trial (DCCT)

[4] and its follow up study the Epidemiology of Diabetes Intervention and Complications (EDIC) [5] trial have demonstrated that intensive treatment to achieve glycaemic control to as near the non-diabetic range as possible, results in fewer and less severe microvascular and macrovascular complications. In people with type 2 diabetes the benefits of strict glycaemic control on the prevention of microvascular complications was demonstrated in the UK Prospective Diabetes Study (UKPDS) [6]. Hence, if it was not for hypoglycaemia everyone with diabetes potentially could be complication free as the insulin dosage would simply be increased until blood glucose concentrations were within the non-diabetic range. However as the DCCT reported the risk of severe hypoglycaemia increases three-fold as glycaemic control is driven towards the non-diabetic range [7]. Improvements in insulin therapy, methods of administration and patient education have been made since the DCCT trial was conducted (1982-1993), yet hypoglycaemia remains a common side-effect of treatment especially in those with type 1 diabetes and its effects deserve to be researched further.

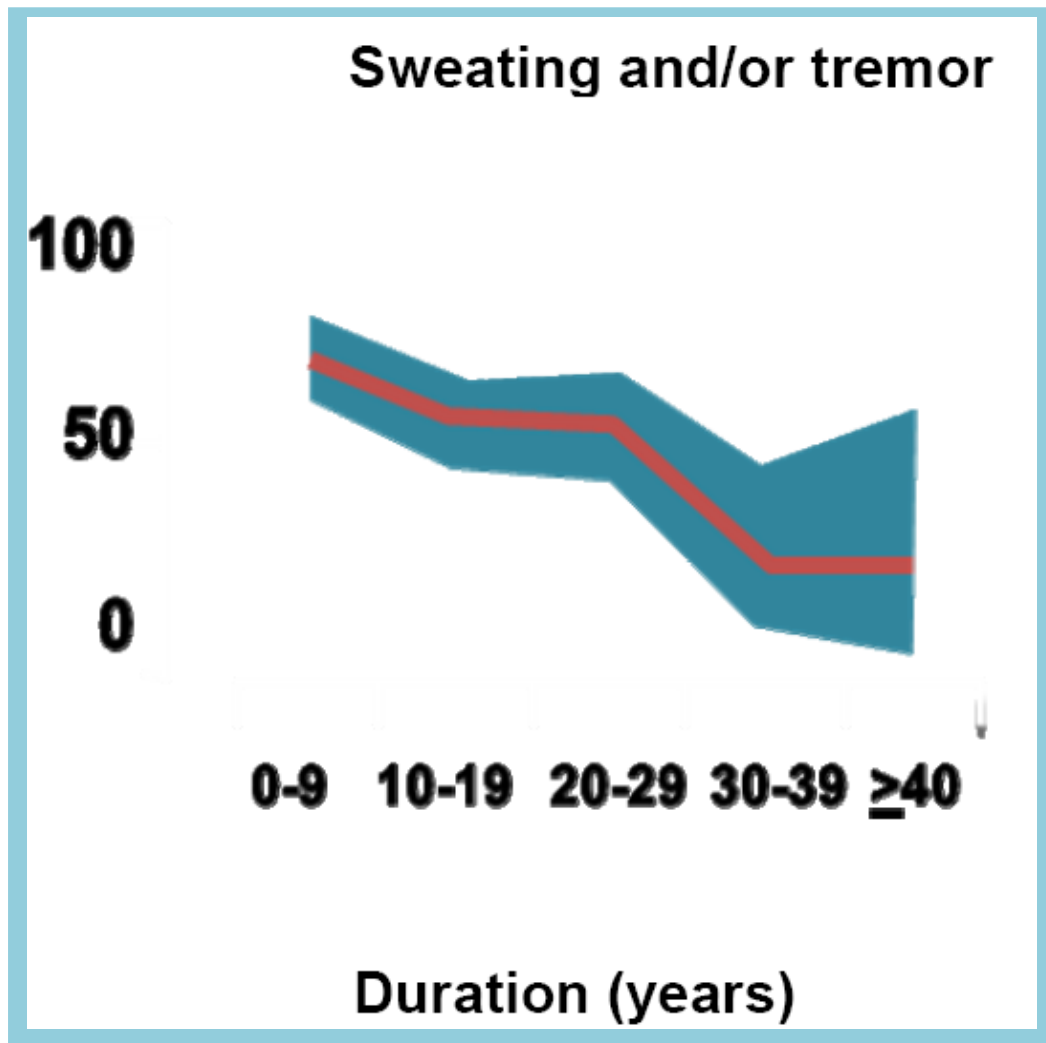
1.2 Defining Hypoglycaemia

Any attempts to quantify the frequency of hypoglycaemia require a precise criterion to diagnose an episode of hypoglycaemia. If Whipples triad [8] - symptoms compatible with hypoglycaemia, a low plasma or blood glucose concentration and resolution of symptoms after glucose concentrations are returned to normal, is considered to be the 'gold standard' for diagnosing hypoglycaemia then this raises difficulties when this criteria is applied to people with type 1 diabetes.

Symptoms of hypoglycaemia are idiosyncratic and age specific and are affected by circumstance. The symptoms of hypoglycaemia are also affected by the duration of

insulin therapy with many people with type 1 diabetes experiencing a reduction in symptom intensity or a change in symptom profile with time [2,9-11]. Neuroglycopenic symptoms such as confusion, drowsiness and an inability to concentrate become predominant, while autonomic symptoms such as tremor, sweating, palpitations etc diminish in prevalence and intensity (Figure 1.1). This leads to the development of “impaired awareness of hypoglycaemia”, which is recognised to be an acquired syndrome associated with cerebral adaptation resulting from recurrent exposure to low blood glucose levels [9]. Hence many episodes of hypoglycaemia can be unrecognised or asymptomatic. In a prospective study of 411 people with type 1 diabetes (65% of whom classified themselves as having normal awareness of hypoglycaemia) Pramming and colleagues reported that when patients had symptoms suggestive of hypoglycaemia only 29% of such episodes were accompanied by evidence of biochemical hypoglycaemia (< 3.0 mmol/L) [2]. Thus solely relying on symptoms appears to be a relatively insensitive method for picking up episodes of hypoglycaemia.

Figure 1.1: Changes in symptoms of hypoglycaemia with increasing duration of diabetes. Adapted from [2]

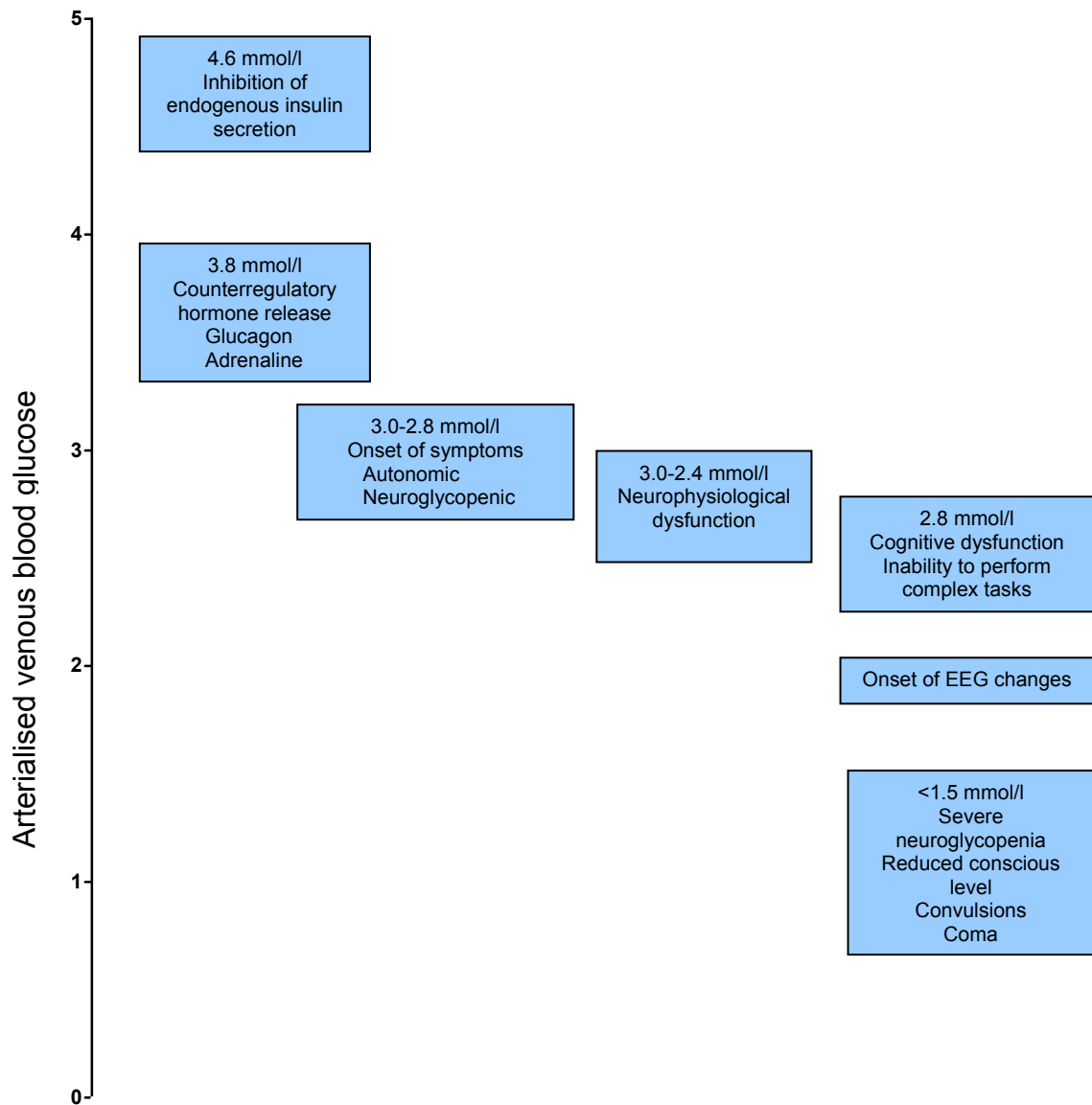


The biochemical definition of hypoglycaemia seems to raise just as many problems as attempts to define hypoglycaemia by symptomatic responses as described above. Hypoglycaemia provokes a hierarchy of events that occur at individual glycaemic thresholds, commencing with counterregulation (arterialised venous blood at ~ 3.6 - 3.9 mmol/L), onset of symptoms (~ 3.0 - 2.8 mmol/L) and cognitive dysfunction (~ 2.8 - 2.4 mmol/L). These glycaemic thresholds are reproducible in non-diabetic individuals [12,13] (Figure 1.2), but are dynamic in people with diabetes and can be altered by external factors such as glycaemic control or recent preceding (antecedent) hypoglycaemia. Patient with poor preceding control may experience symptoms of hypoglycaemia at venous plasma glucose concentrations substantially in excess of 3.0 mmol/L [14]. Whilst patients with strict preceding control may not experience symptoms until venous plasma concentrations have fallen below 2.0 mmol/L [15]

In routine clinical practice low blood glucose concentrations that require treatment should be set on an individual basis however, a generic lower blood glucose concentration of <4.0 mmol/L has been advised by both the American Diabetes Association and Diabetes UK [16,17] .

Finally, the type of blood in which glucose is measured needs to be considered. In routine clinical practice it is the norm for venous blood glucose concentrations to be measured. Arterial blood then ultimately capillary blood supplies glucose to tissues hence arterial plasma glucose concentration is the most accurate assessment of the actual blood glucose concentration. However as arterial sampling is invasive and can be painful venous concentrations are more often used in clinical practice, although these tend to be lower than the arterial samples [18].

Figure 1.2: Hierarchy of endocrine, symptomatic and neurophysiological responses to acute hypoglycaemia in non-diabetic subjects. Adapted from Textbook of Diabetes, 2nd edition, 1997 (Eds Pickup J and Williams G).



1.2.2 Definition of severity of hypoglycaemia

The American Diabetes Association Workgroup on Hypoglycaemia [17] has classified hypoglycaemic episodes as:

- Severe hypoglycaemia - an event requiring assistance of another person to actively administer carbohydrate, glucagon or other resuscitative actions.
- Documented symptomatic hypoglycaemia – an event during which typical symptoms of hypoglycaemia are accompanied by a measured plasma glucose concentration < 3.9 mmol/L.
- Asymptomatic hypoglycaemia – an event not accompanied by typical symptoms of hypoglycaemia but with a measured plasma glucose concentration of < 3.9 mmol/L.
- Probable symptomatic hypoglycaemia – an event during which symptoms of hypoglycaemia are not accompanied by a plasma glucose determination (but that was presumably caused by a plasma glucose concentration of < 3.9 mmol/L).
- Relative hypoglycaemia – an event during which the person with diabetes reports any of the typical symptoms of hypoglycaemia, and interprets those as indicative of hypoglycaemia, but with a measure plasma glucose concentration of > 3.9 mmol/L.

However in general terms most episodes are classified as mild if they can be self-treated or severe if third party assistance is required.

1.2.3 Conclusions regarding hypoglycaemia definition

Given the inherent limitations of relying on symptoms only to diagnose hypoglycaemia and the difficulties surrounding choosing an appropriate plasma glucose for which to define hypoglycaemia in people with type 1 diabetes not surprisingly there is no current consensus on how it should be defined. In an ideal world patients would perform home blood glucose monitoring to document a glucose concentration < 4.0 mmol/L when they perceive themselves to be hypoglycaemic then treat this appropriately. In reality this rarely happens and patients simply self-treat which ultimately is the safest mode of action.

Many have argued that the plasma glucose concentration of < 4.0 mmol/L as suggested by Diabetes UK and the ADA [16,17] to define hypoglycaemia is too high given the fact that at this level it would have no effect on symptom generation, hormonal responses or affect cognitive function and have suggested that a concentration of 3.5mmol/l to define the onset of hypoglycaemia would be more clinically relevant [19].

1.3 Frequency of hypoglycaemia

The frequency of hypoglycaemia in people with type 1 diabetes is difficult to estimate accurately. Recall of mild hypoglycaemia, unlike severe hypoglycaemia which has been shown to be robust for upto year after the event [20], is generally unreliable after one week [2]. Therefore to accurately document the frequency of hypoglycaemia this should be attempted prospectively.

Current available methods for assessing this frequency include home blood glucose monitoring (HBGM) and continuous glucose monitoring (CGMS). Home blood glucose monitoring has its limitations in that the frequency of hypoglycaemia is dependent on the frequency and timing of testing. Thus it is likely that even if the person with type 1 diabetes is checking the recommended four times a day, any inter-prandial, and in particular nocturnal, hypoglycaemic event may be missed and may therefore cause underestimation of the true frequency. Therefore the introduction of CGM whereby an interstitial glucose value is measured every 3 minutes for a period of up to 72 hours would appear to circumvent these problems and be the “gold standard” for estimating hypoglycaemia frequency in people with type 1 diabetes. Concerns however have been expressed about the accuracy of CGM, particularly at hypoglycaemic levels [21] because of the physiological delay between blood and interstitial glucose which may be exaggerated when blood glucose is falling rapidly [22]. In patients with type 1 diabetes during experimental hypoglycaemia, CGM has been proven to underestimate interstitial tissue glucose concentrations [23] and potentially overestimate the frequency of hypoglycaemia.

1.3.1 Frequency of mild hypoglycaemia

Comparing studies of hypoglycaemia frequency is problematic due to variations in study design, heterogeneity of populations and differing definitions of hypoglycaemia confounding comparisons. The studies that have examined this frequency have reported widely varying rates of hypoglycaemia at between 8 to 160 episodes per patient per year and will be examined in detail below.

Retrospective studies

Pedersen-Bjergard and colleagues in two large retrospective studies (2001 & 2004) of 201 and 1076 patients respectively, documented patient recall of mild, symptomatic hypoglycaemia, during the preceding week, at two episodes per week [24,25]. Both studies included a high percentage of participants on intensive insulin regimens and had a similar glycaemic control (HbA1c 8.6%).

Prospective studies

Prospectively collected data on the frequency of hypoglycaemia should theoretically provide more accurate data but again the reported rates vary widely. Pramming and colleagues in a study performed in 1990 (which confirmed the validity of patient recall of mild hypoglycaemia for up to one week) of 411 people with type 1 diabetes, reported an average of 1.8 episodes of mild symptomatic hypoglycaemia per week [2]. Limitations of this earlier study are that 78% of participants were managed on twice-daily soluble and isophane insulin's thus making it less relevant today due to the widespread use of intensive insulin regimens and insulin analogues.

Pedersen-Bjergard and colleagues also examined the frequency of mild hypoglycaemia prospectively in 2003 [26]. Questionnaires were sent monthly for 12 months to participants in order for them to record all episodes of mild symptomatic hypoglycaemia. This occurred on average 1.7 times per patient per week. The results of the studies reported by Pederson-Bjergard and the earlier study of Pramming and colleagues have to be interpreted with a degree of caution. All of these studies reported a high percentage (up to 50%) of participants with impaired awareness of hypoglycaemia. Such a high percentage of participants at a significantly higher risk of

hypoglycaemia will therefore over estimate the true frequency. Questions however have been raised about the validity of the Danish method of assessing awareness of hypoglycaemia and will be discussed in Chapter 7.

In a prospective study from Dundee, Donnelly and colleagues examined the frequency of mild symptomatic hypoglycaemia in 94 people with type 1 diabetes (49% male, median age 40 years with a median duration of diabetes 18.1 years) [27]. Rates of mild symptomatic hypoglycaemia was reported as 42.89 episodes per patient per year. This reported rate is roughly half the rate reported by Pramming and Pedersen-Bjergard *et al* which maybe due to the atypical groups recruited in the Danish studies as discussed above. The study from Dundee also has some limitations: namely the criteria for defining hypoglycaemia was not described and there appeared to be no standard set for the frequency of HBGM as “patients were not required to deviate from their normal routine of blood glucose monitoring”, which is ultimately going to affect the reported frequency.

In a study from our centre which examined the frequency and severity of hypoglycaemia in the workplace, 243 adults were examined prospectively over a 12 month period [28]. This study reported a frequency of mild symptomatic hypoglycaemia of only eight episodes per patient per year, significantly below that previously reported for people with type 1 diabetes. The group of people studied was atypical from the normal clinic population for several reasons. The study reported a very low prevalence of impaired awareness of hypoglycaemia at only 3% in the group of 243 people with insulin-treated diabetes who were in full-time employment. The participants themselves were mainly ‘white collar’ workers in management and professionals with few people undertaking unskilled or manual work. The mean HbA1c was also high at 9.1%. A small percentage

of people with insulin treated type 2 diabetes were also included (11%). Thus all of the above namely the low percentage of participants with impaired awareness, the poor glycaemic control and the small number of subjects with type 2 diabetes could have lowered the frequency of hypoglycaemia.

The UK Hypoglycaemia Study group consisted of six secondary care centres (of which Edinburgh was one) and examined the frequency of hypoglycaemia in type 1 and type 2 diabetes [29]. In total 107 subjects with type 1 diabetes were recruited. Their baseline demographics are below in Table 1.1. All participants were provided with similar capillary glucose testing devices (Medisense G glucose meter, Abbott Laboratories, Abbott Park, IL, USA) and were requested to perform one four-point blood glucose profile daily, (three measurements before meals and one at bedtime) in a 24 hour period, once a week for the duration of the study (12 months). Participants were contacted monthly by members of the research team to collect both the BGM and self-reported hypoglycaemia data. This group reported a frequency of mild hypoglycaemia in those with type 1 diabetes < 5 years of 35.5 episodes per patient per year. In those with duration > 15 years rather surprisingly the reported rate was lower at 29.0 episodes per patient per year. If we exclude the study by Leckie and colleagues (due to the difficulties of transferring data obtained from an atypical group recruited specifically for a study examining hypoglycaemia in the workplace) then this study has the lowest reported rate of mild hypoglycaemia. This study had numerous strengths: a prolonged follow-up period and was conducted at multiple centres throughout the United Kingdom. However this prolonged follow up may have lead to 'patient fatigue' with patients becoming less inclined to report episodes as the study progressed.

Table 1.1: Baseline characteristics of the participants with type 1 diabetes in the UK Hypoglycaemia Study, [29].

	Type 1 < 5 years, n= 50	Type 1 > 5 years, n= 57
Sex (% male)	70.0	57.9
Age (years)	41.3 (12.7)	53.2 (10.4)
BMI (kg/m ²)	26.1 (4.3)	27.9 (4.9)
Duration of diabetes (years)	3.0 (1.3-3.8)	29.8 (21.5-40.3)
Baseline HbA _{1c}	7.3 (1.02)	7.8 (0.73)
1 year HbA _{1c}	7.3 (1.16)	7.6 (0.85)
Fasting C pep (nmol/l)	0.37 (0.17-0.58)	0.09 (0.05-0.26)
Post-glucagon C peptide (nmol/l)	0.45 (0.25-0.69)	0.09 (0.05-0.26)
Hypo awareness score (1-7)	1.85(1.3)	2.97 (1.9)

1.3.2 Frequency of severe hypoglycaemia

The frequency of severe hypoglycaemia reported in studies is more consistent than that of mild hypoglycaemia presumably due to the profound effect that each episode has on the patient, thus providing a more robust end point. Recall of such events have been demonstrated to be robust for up to a period of one year [20].

In population surveys, estimates of the incidence of severe hypoglycaemia in type 1 diabetes range from 1.0 to 1.7 episodes per person per year, with annual prevalences of 30 to 40% [25,30-32]. Table 2 describes the larger studies that have examined this, out with clinical trials in unselected groups of individuals with type 1 diabetes. Intervention trials such as the Diabetes Control and Complications Trial reported lower frequencies of severe hypoglycaemia at 0.19 (conventional treatment group) and 0.62 (intensive) episodes per year, which must be at least partly due to the highly motivated patient group of young, healthy individuals of above average intelligence, who had type 1 diabetes of short duration [7]. Patients with two or more episodes of severe hypoglycaemia in the preceding two years were also excluded from the DCCT based on the feasibility study of 278 participants. Participants in the intensive cohort were also seen on a monthly basis by a diabetes doctor, nurse, dietician and psychologist, which would not be feasible in normal clinical practice.

Severe hypoglycaemia can lead to total amnesia and hence as only one in ten of all episodes of severe hypoglycaemia result in contact with the emergency services, as demonstrated by Leese and colleagues in Dundee [33], verification of these episodes should be sought by not only the patient but also relatives.

Table 1.2: Data from studies examining the frequency of severe hypoglycaemia (requiring third party assistance) in adults with type 1 diabetes.

Study	Participants	Type of study	Follow up	HbA1c	Frequency (per/pt/yr)
Pramming et al 1991	411	Prospective	1 week	8.7	1.4
MacLeod et al 1993	600 (56 with T2DM)	Retrospective	12 months	10.7 (A1)	1.6
Mulhauser et al 1998	684	Retrospective	12 months	8.0	0.21
Ter Braack et al 2000	195	Retrospective	12 months	7.8	1.5
Pedersen-Bjergaard et al 2001	207	Retrospective	24 months	8.6	1.1
Pedersen-Bjergaard et al 2003	170	Prospective	12 months	8.4	1.1
Pedersen-Bjergaard et al 2004	1076	Retrospective	12 months	8.6	1.3
Leckie et al 2005	243 (27 with T2DM)	Prospective	12 months	9.1	0.98
UK Hypo Group 2007	107	Prospective	12 months	7.6	0.34

1.4 Causes of hypoglycaemia

Causation of hypoglycaemia is often multi-factorial and no definite cause can be identified in many episodes. Common causes are discussed below.

Patient errors: On a daily basis a person with Type 1 diabetes has negotiate their blood glucose in order to avoid hyper and hypoglycaemia. Inaccurate calculation of the amount of insulin required for a meal, delayed or even missed meals can lead to hypoglycaemia. This is exacerbated not only by the content of the meals affecting the absorption rate [34,35] but also the intra-individual variability in insulin absorption [36] .

Exercise: In non-diabetic humans moderate exercise results in a 40-50% reduction in circulating concentrations of insulin, below pre-exercise levels [37]. In people with type 1 diabetes exercise either needs to be accompanied by a reduction in exogenous insulin prior to exercise or an increase in the consumption of carbohydrate. The absorption of insulin is accelerated if exercise commences shortly after the insulin injection (particularly when the insulin has been injected into an exercised limb, such as the leg), and enhances the risk of hypoglycaemia. An acute increase in insulin sensitivity also occurs post exercise increasing the risk of delayed hypoglycaemia [38] .

Renal Failure: Insulin requirements are lower in people with advancing renal failure. As their metabolic clearance of insulin is reduced an increased insulin activity is observed. People with renal impairment (raised serum creatinine) have a five-fold higher incidence of severe hypoglycaemia compared to matched subjects with normal kidney (creatinine) function [39].

Co-existent endocrine disease: Endocrine disorders that result in cortisol deficiency, such as Addison's disease and hypopituitarism, due to a decrease in the concentration of counterregulatory hormones, are associated with a higher frequency of hypoglycaemia.

Malabsorption and gastroparesis: Conditions, such as Coeliac Disease (which more common in people with type 1 diabetes) can generate nil, non-specific or mild symptoms making it a potentially difficult diagnosis to make. If however a patient with Type 1 diabetes has unexplained episodes of hypoglycaemia, Coeliac serology should be performed especially if the patient is losing weight. Autonomic neuropathy can result in delayed gastric emptying or frank gastroparesis thus affecting the balance of carbohydrate and insulin absorption promoting hypoglycaemia[40] .

Factitious hypoglycaemia: Insulin is a powerful tool in order to manipulate blood glucose concentrations. Deliberate induction of hypoglycaemia appears to be rare and can be often difficult to detect or confirm. It should be suspected if repeated severe hypoglycaemia occurs with no obvious cause.

Drugs and toxins: Alcohol suppresses hepatic glucose output, increasing the risk of nocturnal hypoglycaemia. Alcohol also impairs the awareness of hypoglycaemia and hinders the individual's ability to take corrective action and prevent progression of mild to severe hypoglycaemia. Up to 19% of all episodes of severe hypoglycaemia necessitating hospital admission have implicated alcohol ingestion [41]. Some drugs, including ACE inhibitors and disopyramide, also have been linked to increased insulin sensitivity [42,43].

1.5 Risk Factors for Severe Hypoglycaemia

Episodes of severe hypoglycaemia can result in accidents, coma, and even death. Not surprisingly it discourages patients and health care providers from pursuing intensive glucose control. The DCCT concluded that only about 8% of future severe hypoglycaemic episodes could be predicted from known variables [7], and a recent structural equation model accounted for 18% of the variance in SH using history of SH, hypoglycaemia awareness, and autonomic symptom score [44]. Therefore current researchers have been looking past traditional risk factors in an attempt to try and predict those at risk of severe hypoglycaemia, and those along with the role of traditional risk factors will be discussed below.

Intensive insulin therapy

The Diabetes Control and Complications Trial Research Group demonstrated that strict glycaemic control decreased the incidence of microvascular complications in people with type 1 diabetes [4]. One thousand four hundred and forty one people with type 1 diabetes were randomised to either intensive insulin therapy (with multiple daily insulin injections or pumps, frequent blood glucose monitoring, monthly visits and frequent telephone advice) or conventional insulin therapy (one to two injections per day, infrequent blood glucose monitoring and three monthly visits. After a follow-up period of approximately 6.5 years the mean HbA1c in the intensive group was 7.0% compared to 8.8% in the conventional group. The incidence of severe hypoglycaemia in the intensive group was three times that compared to the conventional group (0.62 versus 0.19 episodes per patient per year [7]. Similar results were seen in the Stockholm Diabetes Intervention Study of 102 patients with type 1 diabetes randomised to

intensive or conventional treatment groups [45]. Rates of severe hypoglycaemia were again almost three-fold higher in the intensive group (1.1 versus 0.4 episodes per patient per year). Other studies have managed to improve glycaemic control but not increase the risk of severe hypoglycaemia via structured in-patient education programmes prior to intensification of insulin therapy.

Previous severe hypoglycaemia

The main risk factor for severe hypoglycaemia in the DCCT was a history of previous severe hypoglycaemia, although this only accounted for a variance of 5 % in SH risk [7].

Defective Counterregulation

In people with type 1 diabetes, the relative insulin excess from exogenous insulin leads to a fall in glucose concentrations. As the glucose concentrations fall, the plasma insulin levels cannot decrease as observed in people without diabetes. Thus the first line of defence against hypoglycaemia is lost. With falling glucose concentrations the concentration of glucagon should also increase but this response is diminished early in the course of type 1 diabetes, and within a few years becomes negligible or non-existent. Patients who do not mount a glucagon response to hypoglycaemia can secrete glucagon in response to arginine infusion [46]. This paradox appears to have been explained recently by the 'intra-islet hypothesis', which postulates that glucagon release in response to hypoglycaemia also requires a fall in intra-islet insulin concentration, and hence a reduction in tonic α -cell inhibition [47]. In type 1 diabetes, intra-islet insulin concentrations are close to zero at all times due to endogenous insulin deficiency and no such fall is possible. Therefore the third line of defence, the epinephrine response becomes vital. In people with type 1 diabetes this response however is usually

attenuated with antecedent hypoglycaemia shifting the thresholds to a lower level [48-50]. The loss of all three lines of defence against hypoglycaemia substantially increases the risk of hypoglycaemia.

Impaired awareness of hypoglycaemia

With increasing duration of treatment with insulin many people with type 1 diabetes experience a change in their symptoms of hypoglycaemia [2, 9-11], manifested as either a reduction in intensity or number, or a change in symptom profile, so that neuroglycopenic symptoms predominate, while autonomic symptoms are less prominent or absent. This diminished ability to perceive the onset of hypoglycaemia (impaired awareness of hypoglycaemia (IAH), is alleged to affect approximately 20-25% of people with type 1 diabetes [51-53]. Studies in people with impaired awareness have documented a 3-6 fold increase in the risk of severe hypoglycaemia compared to those with intact awareness [51-53].

Hypoglycaemia –Associated Autonomic Failure

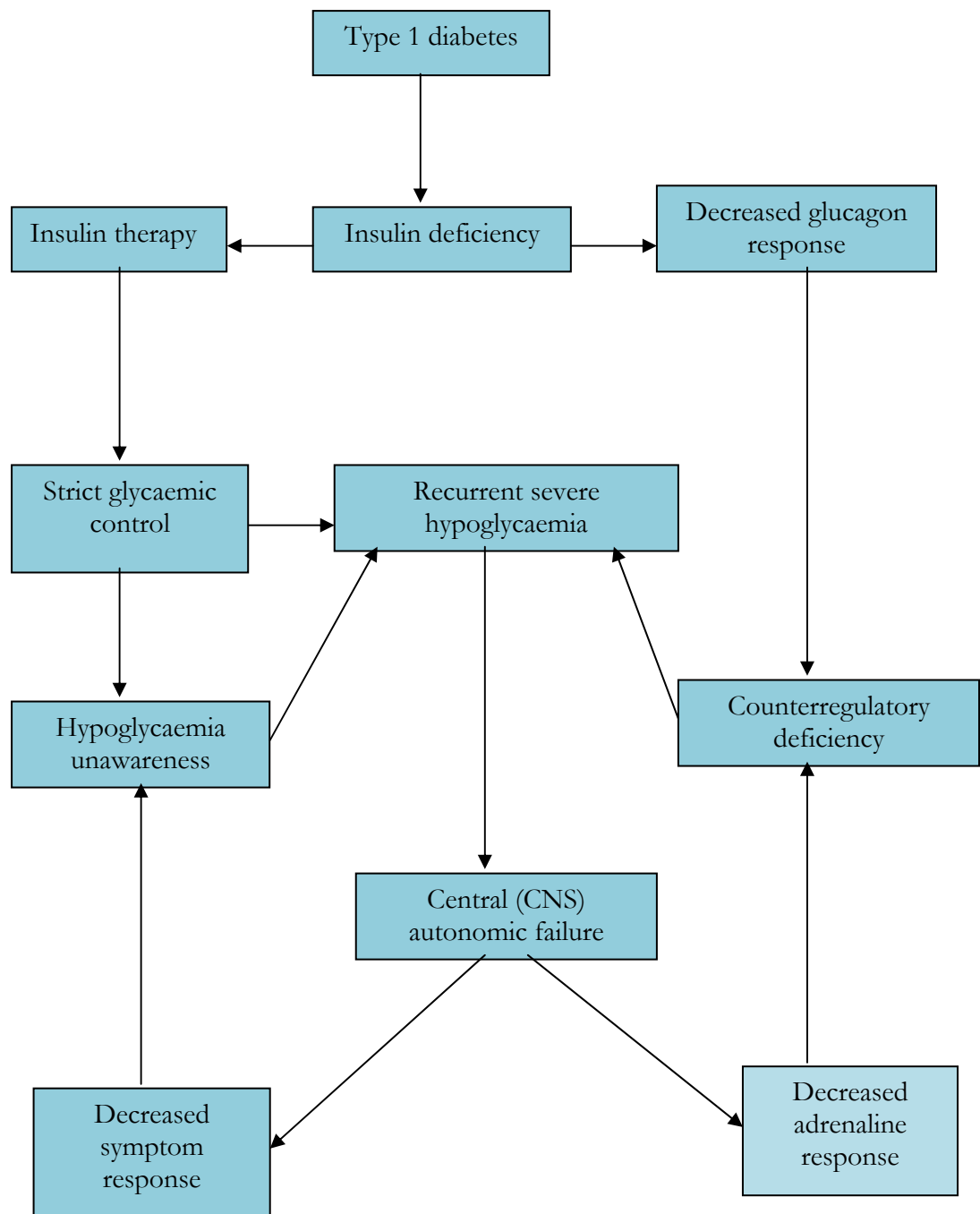
Hypoglycaemia Associated Autonomic Failure (HAAF) has been postulated to occur in both type 1 diabetes and advanced insulin-treated type 2 diabetes, the concept being that recurrent hypoglycaemia results in a failure of the centrally-mediated sympatho-adrenal response which promotes counterregulatory failure and impaired awareness of hypoglycaemia (Figure 1.3), [47].

Low blood glucose index

The low blood glucose index (LBGI) is a measure of the frequency and extent of low home blood glucose monitored values. This validated measure takes into account the

specific distribution of blood glucose data [54-56] as in people type 1 diabetes as it is substantially asymmetric, with the hypoglycaemic range (<3.9 mmol/L) numerically much smaller than the hyperglycaemic range (>10 mmol/L). As a result, standard statistics, such as the mean and SD, tend to underestimate patients' risk for hypoglycaemia. The LBGI is a non-negative quantity that increases when the number and/or the absolute extent of low blood glucose readings increase. The LBGI is also not influenced by hyperglycaemia (all readings above 6.25 mmol/L have zero loads) unlike the mean and SD. Thus LBGI has been proven to predict severe hypoglycaemia better than any other standard statistic with the LBGI accounting for between 40-48% variance of SH risk [56,57].

Figure 1.3: Diagrammatic representation of the concept of hypoglycaemia-associated autonomic failure. Adapted from [47].

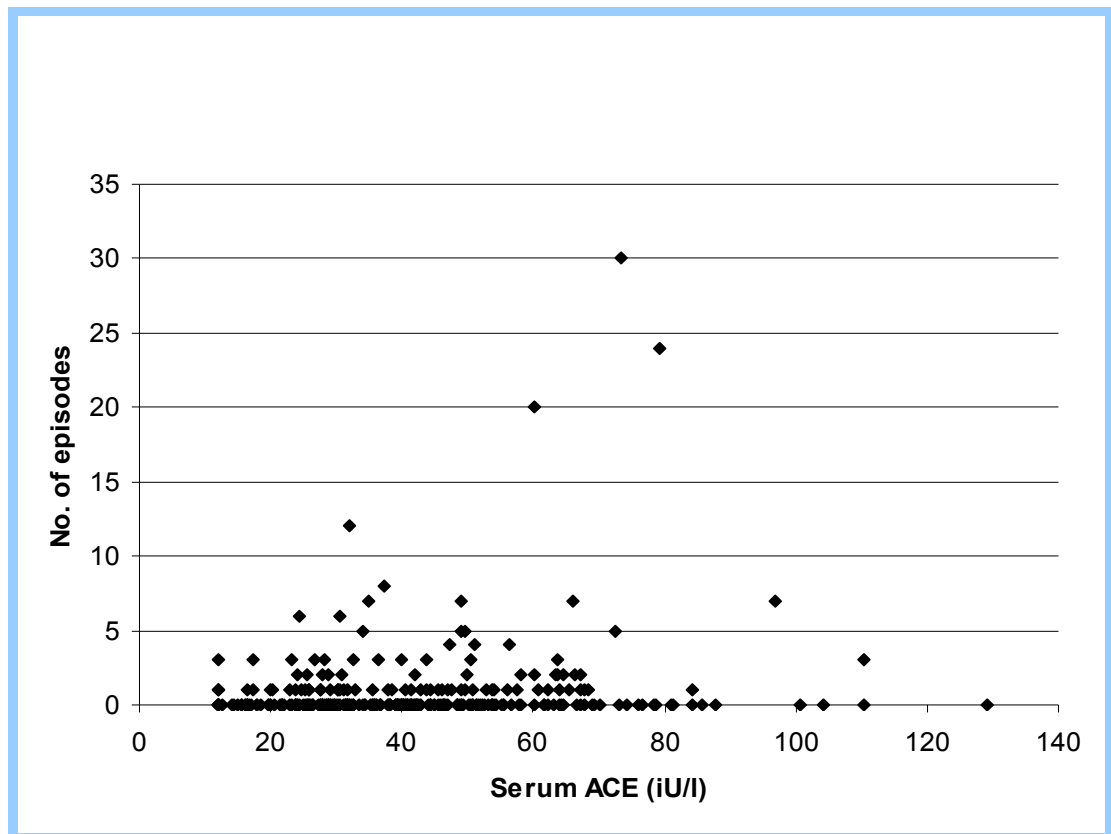


Genetic variation

Serum angiotensin-converting enzyme (ACE) activity has emerged as a possible marker for risk assessment. Individual variation in serum ACE levels is mediated in part by gene polymorphism, via I (insertion) and D (deletion) alleles. The II genotype is associated with low serum ACE activity [58] and in type 1 diabetes has been linked to a low frequency and risk of severe hypoglycaemia; the DD genotype is associated with higher serum ACE activity which has been suggested to be an index of an increased risk of SH [23,25]. Low serum ACE and the II genotype are associated with enhanced athletic performance in events requiring stamina, and have a higher than normal prevalence in high altitude mountaineers [59-61]. A hypothetical explanation for these findings is that a lower ACE activity confers greater ability to function efficiently during periods of metabolic substrate deprivation. Conversely, those who have a high ACE activity have more limited functional capacity when challenged by glucose deficiency.

In people with type 1 diabetes with high ACE activity, this may be manifest by greater cognitive impairment during hypoglycaemia than in those with low ACE activity. This difference in the capability of the brain to function during glucose deprivation, might explain the variable risk of developing severe hypoglycaemia within a population with type 1 diabetes. Two Danish studies in adults, and one Swedish study in children and adolescents, all with type 1 diabetes, have demonstrated that a high serum ACE activity is associated with an increased risk of SH [24,26,62]. However a study from our own centre in Edinburgh of 300 adults with type 1 diabetes failed to demonstrate a convincing association between ACE concentrations and severe hypoglycaemia (Figure 1.4), [63] .

Figure 1.4: The relationship between number of episodes of severe hypoglycaemia (SH) experienced by individual participants during the previous year and their serum Angiotensin Converting Enzyme (ACE) levels. Adapted from [63].



1.6 Treatment of hypoglycaemia

Mild hypoglycaemia is usually self-treated with oral carbohydrate. Guidelines exist for the self-treatment of mild symptomatic hypoglycaemia, and suggest the initial consumption of roughly 20 grams of fast-acting carbohydrate, preferably as glucose (e.g. 3 dextrose tablets) followed by longer-acting carbohydrate in the form of starch (banana, cereal or biscuits). A glucose gel preparation can be applied to the buccal mucosa (although jam and honey work equally well) if the patient's conscious level is high enough, due to the risk of aspiration with a decreased level of consciousness. All forms of refined sugar take on average 15 minutes to relieve symptoms. While the symptoms are persisting during this period many patients over-treat their hypo leading to rebound hyperglycaemia. Unconscious patients should receive 50% dextrose as an intravenous bolus of 20-50 ml (although this concentration may provoke thrombophlebitis and many advocate a larger bolus of 10-20% Dextrose) and, if hypoglycaemia is protracted, an intravenous infusion of 10-20% dextrose may be required to maintain euglycaemia. Intramuscular or intravenous glucagon may also be given to stimulate conversion of glycogen into glucose, but if the hypoglycaemia has been precipitated by, or is associated with, excessive alcohol intake, glucagon may be ineffective as alcohol blocks the glycogenolytic action of glucagon to convert hepatic glycogen into glucose. If the patient has a prolonged episode of hypoglycaemia glucagon will also be ineffective as hepatic glycogen will have been exhausted. Therefore this needs to be given early in the treatment of hypoglycaemia.

1.7 Prevention of hypoglycaemia

Education of people with diabetes must include advice on not only how to recognise and treat hypoglycaemia but also on how to prevent it. Current strategies are discussed below.

Insulin analogues and alternative regimens

Short-acting insulin analogues, which have a rapid onset and short duration of action, are effective in reducing the incidence of hypoglycaemia in people with type 1 diabetes [64]. They are of particular benefit in people with unpredictable lifestyles, as they allow greater flexibility in timing and dosage of insulin and timing of meals. The long-acting insulin analogue, insulin glargine, has been shown in some studies to be beneficial in reducing the incidence of hypoglycaemia, particularly at night [64].

The National Institute of Clinical Excellence recent technology appraisal guidance on the use of Continuous Subcutaneous Insulin Infusion (CSII) for diabetes recommends that CSII be reserved for people with Type 1 diabetes when other therapy has been unable to maintain glycated haemoglobin levels $<7.5\%$ without disabling hypoglycaemia [65]. Previous research has demonstrated a dramatic reduction in episodes of severe hypoglycaemia in patients transferring from a basal bolus regimen with human insulin's to CSII [66].

Diabetes education

Dose Adjustment for Normal Eating courses (DAFNE) are outpatient-based programmes modelled on a German 5 day structured inpatient-training programme in

intensive insulin treatment. Participants are taught to match insulin doses to their food choices, while keeping their blood glucose close to normal, hopefully with minimal input from clinicians once they have completed the course. The initial DAFNE trial demonstrated a sustained improvement in glycaemic control at one year without increasing the risk of severe hypoglycaemia [67].

Home blood glucose monitoring

No clear evidence exists to show that frequent home blood glucose monitoring can reduce the occurrence of severe hypoglycaemia. The Low Blood Glucose Index however has been demonstrated to predict imminent (i.e. within 24 hours episodes) of 58-60% of episodes of severe hypoglycaemia using only three values from home blood glucose monitoring [68]. With more readings available the accuracy appears to increase. Unfortunately at present this mathematical equation is not routinely available, although hopefully this can be incorporated into a metered device at some stage. Despite the lack of evidence for HBGM it appears logical to continue to encourage people with insulin treated type 1 and 2 diabetes to continue to check their blood glucose on regular occasions to detect asymptomatic episodes of hypoglycaemia and facilitate adjustments in their insulin dosage.

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Chapter 2

Effect of Hypoglycaemia on the Brain

2.1 Methodical Considerations

Reproducible studies examining the effects of hypoglycaemia on cognitive function did not become feasible till the late 1970's and throughout the 1980s, although the effects of hypoglycaemia on cognitive function were first reported in the 1920s after insulin was discovered [1]. A considerable volume of literature has accumulated on these effects and will be discussed in detail later. Prior to this however, the methodical differences and limitations of procedures employed in the studies will be examined.

Method of induction of hypoglycaemia

Two main techniques have been used to induce hypoglycaemia in the experimental setting. The insulin infusion technique involves an intravenous infusion of insulin, at a variable rate, to achieve the desired blood glucose concentration. The hyperinsulinaemic glucose clamp technique involves infusing a constant high dose of insulin, to saturate insulin receptors and deliver a maximal hypoglycaemic response, while varying the amount of dextrose infused [2]. The hyperinsulinaemic clamp technique has gained widespread popularity over the past 30 years due to the fact that rapid changes in blood glucose concentrations can be made. Both techniques however have disadvantages. The concentrations of insulin infused vary widely between the two techniques with the clamp technique employing supra-physiological doses and the insulin infusion technique employing variable insulin concentrations.

The human brain is almost entirely dependant on glucose for its energy [3], with a minimal contribution from ketone bodies, amino acids and lactate during the acute state [4]. Transport of glucose into the brain is mediated via glucose transporters (GLUT 1 to 5) as the blood brain barrier prevents simple diffusion across it. Insulin receptors have

been demonstrated throughout the human brain with particularly high concentrations in the hypothalamus, cerebellum and cortex [5]. Pardridge and colleagues have also demonstrated the presence of insulin receptors at the blood brain barrier [6]. Glucose transporters such as the insulin sensitive GLUT 4 transporters and partially insulin sensitive GLUT 1 transporters have been observed at the blood brain barrier brain and on glial cells in various animal studies [7-11]. Therefore, it would appear that the supra-physiological doses of insulin used in the hyperinsulinaemic glucose clamp procedure could potentially improve transport of glucose across the blood brain barrier and thus protect against neuroglycopenia improving cognitive function. Most studies in both humans and animals do not however appear to show an effect of increasing circulating insulin concentrations above that, which is normally found in the fasting state, on glucose transport across the blood brain barrier [12-14]. In a recent study Bingham and colleagues demonstrated that unlike the above research brain glucose uptake is partially insulin sensitive as when insulin is infused at a sub-physiological dose a reduction in brain glucose metabolism was observed [15]

Measurement of blood glucose concentrations

Attention also has to be paid to the nature of the blood analysed i.e. arterial or venous, plasma or whole blood as this will affect the results obtained. Arterial blood sampling remains the “gold standard” but is a painful and difficult procedure to perform. Many studies including those from our own centre, have used arterialised venous blood sampling, where venous blood is taken from a hand that is placed within a heated box to increase blood flow and create a partial arteriovenous shunt [16]. This attempts to minimise the difference between arterial and venous samples.

Plasma glucose concentrations are also 10-20% higher than contemporaneously measured whole blood concentrations [17].

Limitations of neuropsychological testing

Studies of the effects of hypoglycaemia on cognitive function have to utilise neuropsychological tests. There is currently no consensus as to how many cognitive function tests make a minimum appropriate assessment of cognitive function. Is it more appropriate to use one test repeatedly or a battery of tests? The tests themselves such as the commonly used 4 Choice reaction time (4CRT) and Stroop ink colour tasks also measure multiple cognitive domains and not one single domain. The 4CRT for instance requires the domains of attention, visual information processing, central processing and psychomotor function in order to execute the task. Therefore if a deterioration is observed during hypoglycaemia it is impossible to ascertain whether this is due to an impairment in one, some or all domains. The artificial setting of the laboratory also limits the nature of the tests that can be administered as volunteers are either sitting in a chair or lying in a bed with one arm immobilised due to the intravenous infusions of insulin and dextrose. If glycaemic thresholds for cognitive dysfunction are being examined then the tests also have to be quick to administer to allow for serial testing

The transferability of the observed deterioration during hypoglycaemia in certain cognitive function tests to activities of daily living that people with diabetes have to deal with during episodes of hypoglycaemia are also questionable. Some tests such as the driving simulator studies by Cox and colleagues whereby driving performance was impaired by mild hypoglycaemia (3.6 mmol/l in the earlier study and ~3.0 mmol/l in the latter), with a tendency to veer off course, drive too fast and brake inappropriately have an obvious transferability [18,19] . Whereas other tests such as memory, while

important, do not have the same relevance during an episode such as acute hypoglycaemia. Performance in most tests will improve with practice therefore the results of the tests obtained during hypoglycaemia must be compared to those obtained at similar time points during euglycaemia and not simply compared to those obtained at baseline which was an inherent problem in many earlier studies. In addition the studies should be counterbalanced with half of the subjects undergoing the hypoglycaemia session first followed by the euglycaemia session and vice versa.

Finally many moderators of cognitive function exist such as the presence of diabetes, antecedent hypoglycaemia, glycaemic control, baseline IQ will affect performance on tasks and these will be discussed in detail in Chapter 3.

2.2 Cognitive Domains Affected by Acute Hypoglycaemia

There is an extensive literature on the effects of acute hypoglycaemia, but as discussed above differing methodology can make comparing studies difficult, with the reported results varying widely. However a few important conclusions can be drawn. Studies with a higher cognitive load appear more susceptible to neuroglycopenia than simple tasks. Finger tapping, a simple motor task appears to be relatively unaffected by hypoglycaemia in healthy volunteers and in people with diabetes at a blood glucose concentration of 3.1 and 2.0 mmol/L [20-22]. Simple reaction time to either a light or sound appears only to deteriorate below 2.7 mmol/L in non-diabetic volunteers [23-25] but 4 Choice reaction time (with 4 potential responses) is reported to be affected at a blood glucose of 3.2 mmol/L [26,27]. Similar trends have been observed in people with diabetes [20,23,28].

Cognitive function appears to be consistently affected at a blood glucose below 2.9 mmol/L with a deterioration in speed of arthrimetic calculation, verbal fluency, colour identification, trail making, digit symbol test performance, digit span and memory function all reported [29,30]. Non-cognitive effects such as a change in mood with an increase in tense arousal and a decrease in energetic arousal are also reported [31].

Table 2.1 examines the effects of moderate hypoglycaemia in healthy volunteers. Table 2.2 the effect in adults with type 1 diabetes. Studies examining the effects of moderators of cognitive function were not reviewed as these are discussed in Chapter 3. Studies examining between group differences (healthy volunteers versus people with Type 1 diabetes) were also not examined.

The tables are by no means a complete synopsis of this area, as this has been comprehensively reviewed by Deary [29,30].

Table 2.1: Data from studies examining the effects of hypoglycaemia on various aspects of cognitive dysfunction in healthy volunteers

Study	Stevens et al (1989)	Blackman et al (1990)	Kerr et al (1993)	McCrimmon et al (1997)	Evans et al (2000)	McAulay et al (2001)	Sommerfield et al (2003)	Warren et al (2004)
Design	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp
Euglycaemia control arm	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
No of subjects	12	19	10	20	8	20	16	16
Age (years) (Median)	23 (range 18-27)	26.8 (1.3)	Range 20-33	27 (range 21-42)	28.5 (3.5)	28.7 (5.3)	29.6 (2.9)	NR
Cognitive tests	FTT, TMB, DSS, CFFT, CRT, auditory and visual RT	Simple RT, P300	P300 auditory evoked potential	Information processing, TMB, DSS	4 CRT, Stroop, TMB	TEA, RPM	AVLT, LMT, TMB, DSS, BVRT, WMT	RAPM, AH5, TMB, DSS
Glucose Nadir (mmol/L)	3.4 mmol/L	2.6 mmol/L	32.8 mmol/L	2.6 mol/L	2.6 mmol/L	2.6 mmol/L	2.6 mmol/L	2.5 mmol/L
Outcome	Decrease in DSS, TMB	Increase in P300 latency and simple RT	Increase in P300 latency	Decrease in DSS, TMB and Information processing	Decrease in 4CRT and Stoop	Decrease in attention	Decrease in DSS, TMB, Working and delayed memory	Decrease in TMB, DSS and RAPM

Table 2.2: Data from studies examining the effects of hypoglycaemia on various aspects of cognitive dysfunction in people with type 1 diabetes

Study	Holmes et al (1983)	Holmes et al (1984)	Holmes et al (1986)	Pramming et al (1986)	Ewing et al (1998)	Strachan et al (2002)	Sommerfield et al (2003)
Design	Insulin infusion	Insulin infusion	Insulin infusion	Insulin infusion	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp	Hyperinsulinaemic glucose clamp
Euglycaemia control arm	No	No	No	No	Yes	Yes	Yes
No of subjects	12	12	24	16	16	15	15
Age (years)	NR	18-35 years	21.3	28 (Range 20-46)	26.9 (Range 18-47)	26.5 (9.1)	28.5 (20.0-38.2)
HbA1c (SD)	NR	NR	9.6 %	8.7 (Range 7.2-10.1)	8.5 (1.3)	8.8 (2.0)	8.2% (6.9-8.7) (Median (Range))
Duration of Diabetes	NR	NR	8.2	12 (range 4-28)	8.8 (Range 12-17)	11.1 (6.6)	4.5 (1.2-8.4) (Median (Range))
Cognitive tests	AVLT, Digit supraspan, MFFT, RT, BVRT, NDRT, Mathematical computation	Verbal associated fluency, Stoop	Simple RT, Choice RT, letter recognition, FTT	Digit span, serial subtraction, story recall, FTT, categorisation test	Visual information processing test, TMB, DSS	DSS, TMB, AERP, Auditory Information Processing.	AVLT, LMT, TMB, DSST, BVRT, WMT
Glucose Nadir	3.3 mmol/L	3.0 mmol/L	3.0 mmol/L	2.0 mmol/L	2.6 mmol/L	2.6 mmol/L	2.5mmol/L
Outcomes	Decrease in RT and mathematical computation	Decrease in verbal fluency	Decrease in CRT	Decrease in all tests	Decrease in Visual IP, DSS, TMB	Decrease in TMB, DSS and aspects of AP	Decrease in short-term, delayed and working memory

Footnotes to tables 2.1 and 2.2:

AERP = Auditory Event Response Potentials

AH5=Alice Heim 5

AP = Auditory Processing

AVLT = Auditory Verbal Learning Test

BVRT= Benton Visual Reproduction Test

CRT=4 Choice Reaction Time

DSS= Digit Symbol Substitution

FTT = Finger Tapping Test

IP = Information Processing

LMT = Logical memory test

MFFT = Matching Familiar Figures Test

NDRT = Nelson Denny Reading Test

RPP = Ravens Progressive Matrices

RAPM = Ravens Advanced Progressive Matrices

TEA= Tests of Everyday Attention

TMB=Trail Making B

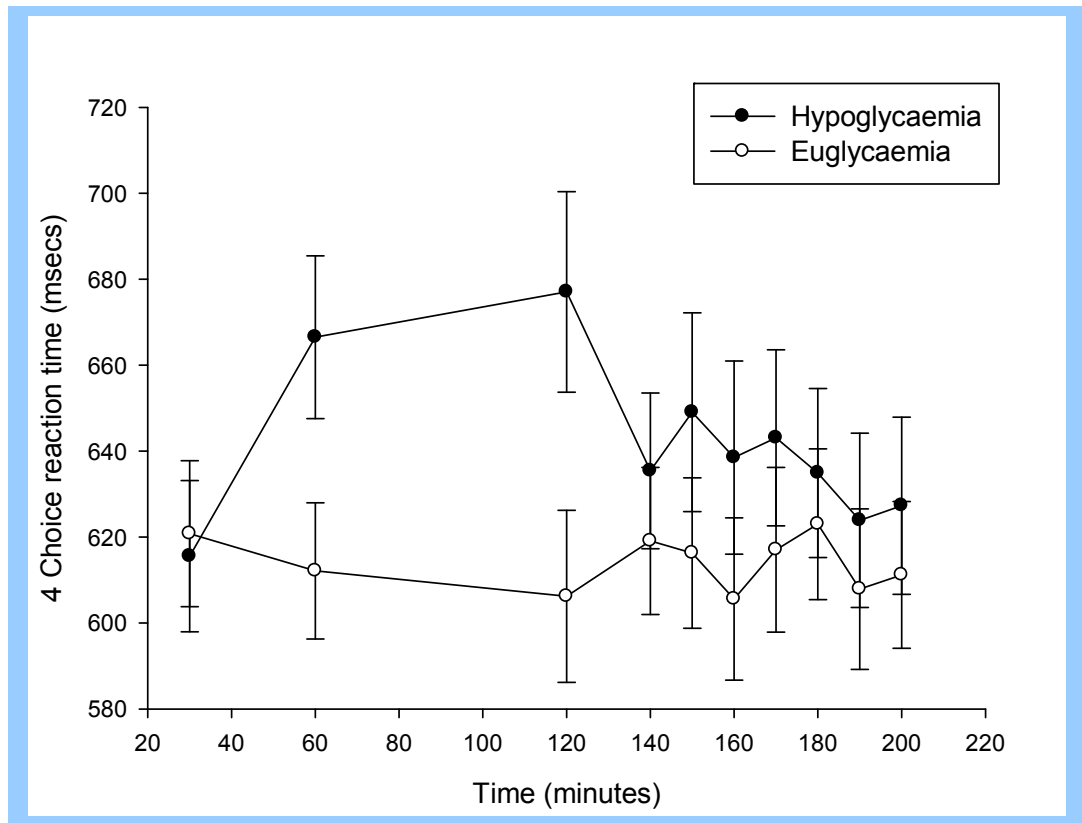
WMT = Working Memory Test.

2.2.1 Recovery of cognitive function after induction of hypoglycaemia

Given the fact that the brain is almost entirely dependent on glucose for its energy, acute hypoglycaemia should lead to an almost instantaneous deterioration in cognitive function and as symptoms are generated centrally, symptomatic awareness of such an event [32,33]. Evans and colleagues induced hypoglycaemia (blood glucose nadir 2.6 mmol/L) in 8 healthy volunteers [34]. After 90 minutes the blood glucose concentration was then rapidly restored. Cognitive function (4 CRT, Stroop and Trail Making B), symptoms and counter regulatory responses were assessed throughout the test period. There was statistically significant impairment of cognitive function immediately, whereas counter regulation and symptoms only achieved significance at 20 minutes. It therefore appears that neuroglycopenia develops even in people with normal awareness of hypoglycaemia before symptoms of low blood glucose develops.

Recovery of cognitive function is generally thought to occur between 45-90 minutes after blood glucose concentrations are returned to normal [23,24,35,36]. The latest study to examine this came from Zammitt and colleagues in Edinburgh [37]. They examined the effect of acute hypoglycaemia (nadir 2.5mmol/L) on recovery of cognitive function in 20 people with normal awareness of hypoglycaemia and demonstrated that a deterioration in choice reaction time persisted for up to 75 minutes after correction of hypoglycaemia (Figure 2.1). Earlier studies whose results have to be interpreted with a degree of caution due to methodical limitations have reported rates of between 20 to 60 minutes [34,38].

Figure 2.1: Mean (SE) times on Choice Reaction Time and Trail Making B during euglycaemia and hypoglycaemia. Adapted from [37].



2.3 Effect of hypoglycaemia on cognitive function

Studies have indicated that people with diabetes have a relatively poorer cognitive ability than matched controls [39,40]. The aetiology behind this is probably multifactorial including chronic hyperglycaemia, recurrent diabetic ketoacidosis, exposure to severe hypoglycaemia, hypertension, cerebrovascular disease etc however most of the research has focused on the role of severe hypoglycaemia.

Strachan and colleagues examined the effect of a single episode of spontaneous severe hypoglycaemia on cognitive function and mood state [41]. Twenty subjects with insulin treated diabetes and a recent hypo were compared to 20 subjects who had not had an episode of severe hypoglycaemia within the preceding year. Subjects were matched for baseline IQ, age, sex and duration of diabetes. Subjects were examined at three time points following the event, on average 1.5, 8.9 and 30 days. The participants completed an extensive battery of cognitive function tests including: NART, WAIS-R, TMB, Forward and backward digit span, logical memory, figural memory, visual change detection, verbal fluency test, PASAT, CRT, Stroop test and various mood and anxiety scores. At the first time point only one (block design) out of the 14 cognitive function tests demonstrated any difference between the two groups suggesting recovery of cognitive function occurs up to 36 hours after the initial event. Although rare single episodes of very severe hypoglycaemia have been reported to lead to permanent damage due to neuronal necrosis [42].

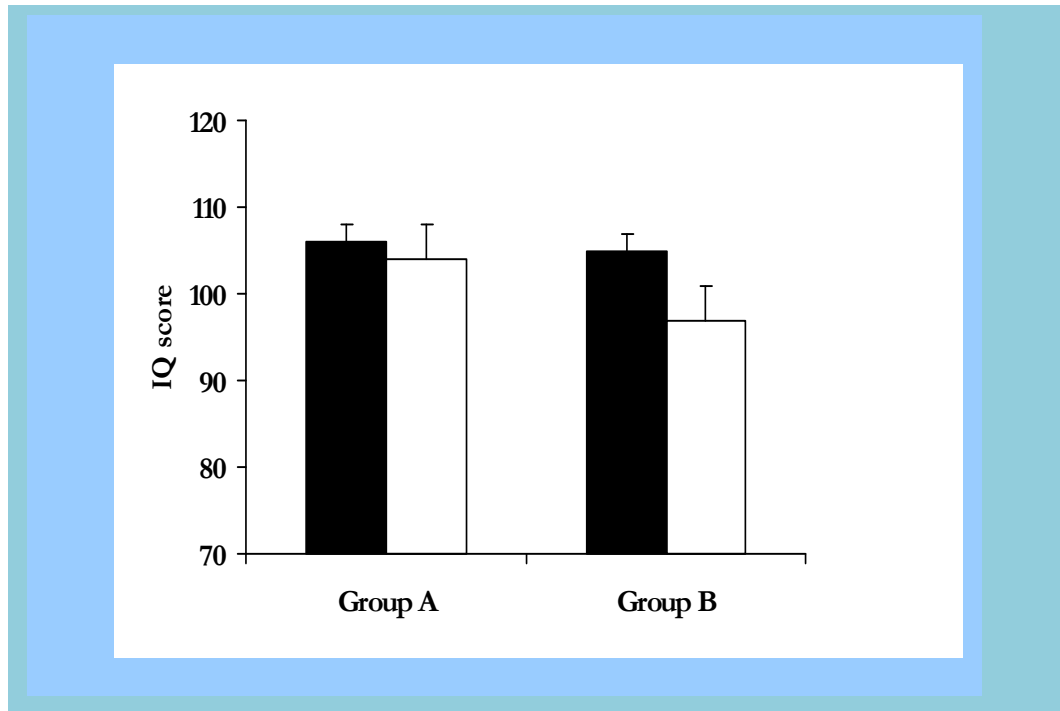
Exposure to recurrent severe hypoglycaemia in retrospective cross-sectional studies has suggested that this leads to cognitive impairment. Wredling and colleagues examined cognitive function in 17 adults with type 1 diabetes and a history of severe hypoglycaemia and compared them to 17 adults with type 1 diabetes and no history of

severe hypoglycaemia. Participants were matched for age, sex, duration of diabetes, educational achievement, employment status and presence of microvascular complications [43]. The cohort with exposure to severe hypoglycaemia performed statistically worse on several cognitive function test including digit symbol. The study design did not preclude the possibility that the patients who had a history of recurrent hypoglycaemia may have been those who had a lower pre-morbid IQ and, being less adept in their self-management of diabetes, had therefore experienced a higher frequency of severe hypoglycaemia. Sachon and colleagues studied 30 patients with insulin dependent diabetes and 30 without a history of severe hypoglycaemia. In addition 25 non-diabetic controls were studied [44]. The patients with previous severe hypoglycaemia performed worse on several cognitive function tests (trail making, verbal fluency and memory) however those with a history of hypoglycaemia were significantly older than those without which could have affected the results obtained. Langam and colleagues from Edinburgh demonstrated a significant correlation between intellectual impairment and history of severe hypoglycaemia in a group of one hundred people with type 1 diabetes [45]. Intellectual impairment was calculated as the difference between pre-morbid cognitive function assessed via the National Adult Reading Test (which correlates highly with IQ and is resistant to the effects of organic brain damage, and thus may represent 'best ever' intelligence) and current performance IQ [46]. Further sub-group analysis, splitting the cohort into quartiles on the basis of their previous experience of severe hypoglycaemia, revealed that those with five or more episodes of severe hypoglycaemia compared to those with no previous severe hypoglycaemia, a significant difference was observed in IQ deficit (approximately 6 IQ points), performance IQ and reaction times, (Figure 2.2). While this study indicated a relationship between the frequency of severe hypoglycaemia and cognitive impairment,

it could not exclude the possibility that diabetes *per se* was affecting cognitive function. In a follow up study by Deary and colleagues the same 100 subjects with type 1 diabetes were compared to a group of 100 non-diabetic controls, matched for sex, age, years of education and social class [47]. No difference was also observed in NART scores and thus pre-morbid IQ. This study demonstrated that the performance and verbal IQs of the diabetic participants were lower than the non-diabetic controls. Once the effects of severe hypoglycaemia were controlled for the difference in performance IQ between the healthy volunteers and subjects with diabetes was abolished. The significant between group differences in verbal IQ persisted after controlling for severe hypoglycaemia suggesting additional factors.

Similar results delineating a correlation between decline in performance IQ and frequency of previous severe hypoglycaemia were observed from a study in Nottingham [48].

Figure 2.2. No significant difference in pre-morbid (black bars) and current IQ (white bars) was demonstrated in Group A (no previous SH) but a significant difference was demonstrated in those with > 5 previous episodes of SH, $p < 0.001$. Adapted from [45].



Cross-sectional studies however have their limitations. Firstly an association can be implied by the results obtained not causality. Secondly between group comparisons can introduce potential cofounders unless the groups are meticulously matched as cognitive function is known to be affected by variables such as age, gender, concurrent medical conditions, medication, alcohol and drug ingestion etc. Thirdly many of the above studies did not match the groups for pre-morbid IQ i.e. best achievable IQ which is preserved even in generalised cognitive decline. Lastly the frequency of severe hypoglycaemia has to be estimated retrospectively and this has been shown to be reliable for up to one year after the event [49]. Therefore the self-reported “lifetime” frequency of severe hypoglycaemia has to be interpreted with caution.

Prospective studies therefore would appear to allow causality to be drawn whilst minimising the confounding variables as subjects are acting as their own controls. The largest prospective trials of type 1 diabetes are the Diabetes Control and Complications Trial (DCCT) [50] and the Stockholm Diabetes Intervention Study (SDIS) [51]. Both of these studies were designed to evaluate the effect of strict glycaemic control on limiting the complications of diabetes. The participants were subdivided into intensively-treated and conventionally-treated subgroups, achieving either strict or moderate glycaemic control respectively, the incidence and progression of diabetic complications and the frequency of adverse effects of treatment were monitored prospectively. Both studies have shown unequivocally that strict glycaemic control limits the development and progression of diabetic microangiopathy, but is accompanied by a higher rate of severe hypoglycaemia.

In the DCCT patients underwent detailed cognitive function testing at entry and at three other points (two, five and seven years later) with an average of 6.5 years. In the SDIS the 102 participants were followed up for an average of 7.5 years but the cognitive

function testing in the SDIS was more limited. In both studies no association between episodes of severe hypoglycaemia and cognitive decline was demonstrated [50,51]. The cognitive evaluation carried out as part of the DCCT was repeated 12 years after the trial ended as part of the Epidemiology of Diabetes Intervention and Complications (EDIC) study [52]. Follow up data was available for 1144 patients (85% of the original cohort) assessed on average 18 years after the start of the trial. Again there was no difference in cognitive function between participants allocated to either conventional or intensive treatment with no association between frequency of severe hypoglycaemia and cognitive change. These results appear to suggest that strict glycaemic control and the almost inevitable severe hypoglycaemia risk that accompanies this leads to minimal or no effect on cognitive function. The participants enrolled into the DCCT as discussed previously are somewhat atypical of the general population with diabetes. They were relatively young and had diabetes of short duration. They were also above average intelligence, were highly motivated and were well educated in self-care of their diabetes. People who had a history of multiple episodes of severe hypoglycaemia were excluded. This criterion is likely to have excluded anyone with impaired hypoglycaemia awareness, so that few, if any, patients were recruited to participate in these studies who were at high risk of developing severe hypoglycaemia, thus not surprisingly the annual rate of hypoglycaemia reported was about half that from studies of unselected individuals with type 1 diabetes. This makes it difficult to translate these results to an average diabetic population containing individuals at high risk of severe hypoglycaemia.

2.4 Effect of hypoglycaemia on brain structure

Profound, protracted severe hypoglycaemia while uncommon can lead to permanent neurological and cognitive deficits [42]. Anecdotal case reports of individuals who suffered from protracted severe hypoglycaemia revealed localised neuroimaging abnormalities predominantly affecting the frontal lobes and deep grey matter [53-59]. In rat studies, exposure to a single episode of severe hypoglycaemia lasting between 10-60 minutes lead to widespread neuronal damage, the extent of which correlated with duration of exposure to hypoglycaemia [53]. The pattern of neuronal damage was not in keeping with that found after exposure to ischaemia and raised the possibility of neuronal damage secondary to release of excitotoxins such as glutamate and aspartate. Potentially therefore exposure to repeat episodes of moderate hypoglycaemia could lead to neuronal damage.

Permanent EEG changes (an increase in frontal slow activity and a decrease in alpha frequency) have been demonstrated in those with recurrent severe hypoglycaemia [60-63]. Acute hypoglycaemia is known to increase cerebral blood flow, particularly to the frontal lobes [64]. MacLeod and colleagues in Edinburgh examined 20 subjects with type 1 diabetes (10 of whom had a history of exposure to severe hypoglycaemia) and 20 age & sex matched healthy volunteers [65]. Cerebral blood flow was assessed by SPECT with ^{99m}Technetium Exametazime. An alteration in the pattern of baseline regional cerebral blood flow was observed in the patients with diabetes with frontal excess and relative posterior reduction in cerebral blood. Results were more pronounced in those with a history of severe hypoglycaemia. Similar results from the same group were demonstrated in those with impaired awareness of hypoglycaemia [66].

Whether or not hypoglycaemia leads to structural changes in the brain is less clear cut. A small study of 11 people with type 1 diabetes and no severe hypoglycaemia and 11 with >5 episodes of severe hypoglycaemia since diagnosis revealed that cortical atrophy was more prevalent in subjects with recurrent severe hypoglycaemia [67]. In another later study from our centre no association was observed between previous severe hypoglycaemia and structural abnormalities of the brain using MRI [68]. Unlike the previous study no relationship was identified between severe hypoglycaemia and cortical atrophy. The later study had significantly more participants (71 compared to 22 in the previous study) and studied a younger population. The main finding was that chronic hyperglycaemia (inferred by background retinopathy) was associated with small punctuate white matter lesions in the basal ganglia and periventricular regions. Those with background retinopathy also performed less well on cognitive function tests suggesting the observed results were secondary to “diabetic encephalopathy”, to which vasculopathy, hyperglycaemia and hypertension may have contributed.

2.4.1 Conclusion

Data from cross-sectional studies in adults with type 1 diabetes have suggested that exposure to recurrent hypoglycaemia may lead to a modest impairment of cognitive ability. Whether this small (a 0.33-0.5 decrease in standard deviation) reported reduction in cognitive ability would translate into deterioration of activities of daily living for people with type 1 diabetes remains unlikely. The two prospective studies (including the EDIC follow up) provide some reassurance that exposure to severe hypoglycaemia will generally on the whole not lead to significant cognitive impairment. There are however large inter-individual differences with some individuals suffering devastating cognitive

dysfunction whilst others are relatively unaffected after exposure to severe hypoglycaemia.

Results from neuroimaging studies again offer confirmation that exposure to severe hypoglycaemia does not on the whole, unless severe and protracted, lead to structural abnormalities of the brain.

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Chapter 3

Moderators of Cognitive Function during Acute Hypoglycaemia in Humans

3.1 Background

Hypoglycaemia frequently occurs in insulin-treated diabetes [1,2], and can impact upon all aspects of life [3]. While hypoglycaemia impairs cognitive function [4], not all cognitive domains are affected equally [5-11]. Considerable inter-subject variability exists in the magnitude of cognitive impairment [9,12-18], and several moderators influence the susceptibility of an individual's cognitive performance to the effects of hypoglycaemia.

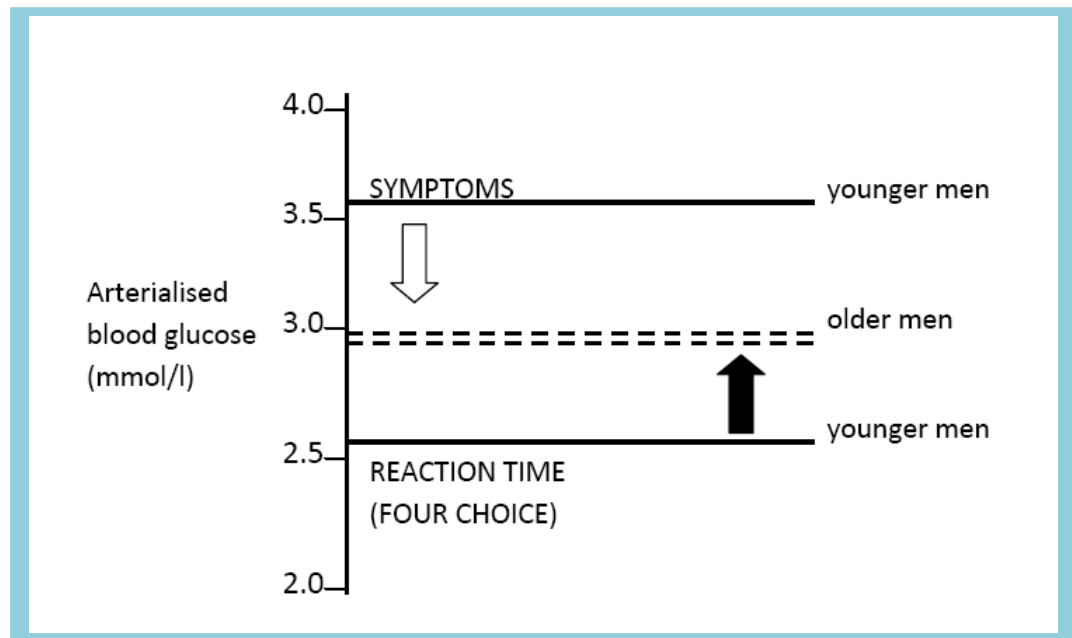
The search for moderators that predict individual vulnerability assumes that this clinical response to hypoglycaemia is consistent across time and equivalent degrees of hypoglycaemia, and that it does not simply reflect individual variation within a population. Gonder-Frederick and colleagues [19] used a battery of cognitive tests to study 26 adults with type 1 diabetes at three levels of blood glucose (6.3, 3.6 and 2.6 mmol/l); the tests were repeated following restoration of euglycaemia. To assess the temporal reliability of individual differences of deterioration in performance during hypoglycaemia, 15 of the subjects were re-tested after three months. Considerable variation was demonstrated between the subjects' responses to hypoglycaemia [19]. At a blood glucose of 3.6mmol/l, 19% of the subjects exhibited a significant deterioration in cognitive performance, while almost half were unaffected, but at 2.6 mmol/L a significant deterioration occurred in more than 50% of the subjects; only 15% exhibited preservation of performance. On subsequent re-testing, individual variations in vulnerability to, and the degree of, cognitive dysfunction, were unchanged, indicating that individual differences to hypoglycaemia are stable and do not occur at random. This suggests that there are stable individual differences in susceptibility to the cognitive effects of acute neuroglycopenia.

The factors that account for the individual differences to hypoglycaemia-induced cognitive dysfunction are of clinical importance in type 1 diabetes and various moderators have been identified.

3.2 Age

Age influences the counterregulatory and symptomatic responses to hypoglycaemia. In older people, the magnitude of adrenaline (epinephrine) and glucagon responses is lower, autonomic symptoms are less profound and neuroglycopenic symptoms predominate [20-24] (Figure 3.1). One small study utilised the four-choice reaction time, a test of co-ordination and psychomotor speed, to examine the effects of ageing on the glycaemic thresholds for cognitive impairment during controlled hypoglycaemia in two groups of non-diabetic men [24], seven aged 60-70, and seven aged 22-26 years. Blood glucose was lowered in a stepwise manner from 5.0 to 2.4 mmol/l, and at each plateau the counterregulatory hormones, symptoms, and four-choice reaction time were assessed. degree. In the younger group, the symptoms of hypoglycaemia commenced at a higher blood glucose (3.6 vs. 3.0 mmol/l) and were more intense.

Figure 3.1: The difference between the glycaemic threshold for subjective awareness of hypoglycaemia and that for the onset of cognitive dysfunction may be absent in the elderly. Adapted from [24].



In the older men, no discernible difference was observed between the blood glucose thresholds for onset of the symptoms of hypoglycaemia and cognitive impairment. Thus the opportunity to detect and self-treat hypoglycaemia before more disabling neuroglycopenia supervened was less in the older subjects. These age-related differences in the glycaemic thresholds for symptom generation and cognitive impairment during hypoglycaemia may put older people at greater risk of severe hypoglycaemia, through inability to identify early hypoglycaemia.

Other studies have shown no consistent correlation between age and the degree of cognitive impairment during hypoglycaemia, but the differences in age between the subjects were modest [17,19] with an upper age limit of 52 years, which may have prevented identification of age-related effects on cognitive function.

3.3 Gender

Significant gender differences in cognitive performance during acute hypoglycaemia and hyperglycaemia were demonstrated in a study of 20 men and 22 women with type 1 diabetes [17] , in whom many cognitive abilities were tested, including sensory perceptual processing, simple motor abilities, attention, learning and memory, language, and spatial and constructional abilities. Patients were examined at plasma glucose levels of 2.2, 5.6 (euglycaemia), 8.9 (baseline), 14.4, and 21.1 mmol/l. All measures of cognitive performance were impaired at 2.2 mmol/l when compared with the baseline performance. The performance decrement of cognitive impairment from baseline (where no gender differences were observed) in the tests, which examined selective and sustained attention (digit vigilance test) and mental flexibility (trail making B), was less in women and this difference persisted after adjustment for other potential confounding factors. No gender differences were observed with tests of other aspects of cognitive function, nor was this discrepancy evident in the cognitive test scores at lesser levels of hypoglycaemia. However these results have to be interpreted with care as only 10 subjects underwent a separate euglycaemia clamp. The indication of a gender difference in cognitive susceptibility to hypoglycaemia is supported by a further study that demonstrated that women performed better on cognitive function testing than men during mild hypoglycaemia (venous plasma glucose 3.6 mmol/l) [19]. However, in this study, the degree of cognitive impairment was similar in both sexes during more profound hypoglycaemia (blood glucose 2.6 mmol/l). The fact that hypoglycaemia appears to cause less cognitive dysfunction in females could potentially explain the lower incidence of severe hypoglycaemia rates in the female participants in the intensively –treated group of the DCCT trial [25].

Paradoxically other studies have shown that women have less intense counterregulatory hormonal response than males, although the glycaemic thresholds at which these responses are triggered are similar in both sexes [26-28]. No difference between the sexes was also reported in the hypoglycaemia symptom scores of 160 subjects, including non-diabetic volunteers and people with type 1 diabetes, in whom controlled hypoglycaemia had been induced [29].

3.4 Intelligence

It has been suggested that highly intelligent individuals possess more 'brain reserve capacity' for cognitive processing, that will confer protection if cognitive function is compromised during cerebral insults such as hypoglycaemia [30]. Gold and colleagues [31] examined whether IQ level exerts a differential effect on hypoglycaemia-induced cognitive dysfunction by studying 24 healthy non-diabetic volunteers, subdivided into groups with high and average IQ. The latter was determined using two standard tests of general intelligence (the Alice Heim 4 test and the National Adult Reading Test). Cognitive function was measured during euglycaemia (plasma glucose 4.5 mmol/l) and during hypoglycaemia (plasma glucose 2.5 mmol/l). At baseline, the high IQ group demonstrated a significantly better performance in most of the cognitive tasks, but during hypoglycaemia cognitive function deteriorated irrespective of IQ, and very few differences were observed between the two groups in performance in the cognitive tasks. Multiple univariate analysis of variance revealed an influence of IQ on the hypoglycaemia effect on two of the cognitive tests. During hypoglycaemia, the group with average IQ deteriorated significantly less than the high IQ group in the tests of attention and information processing (Paced Auditory Serial Addition Test (PASAT)

and Rapid Visual Information Processing). This could have been a type 1 statistical error. These results suggest that individuals with a higher IQ are not protected from the adverse effects of hypoglycaemia on cognitive function, and that they may in fact be more vulnerable to the detrimental effects of neuroglycopenia on intellectual ability.

Studies of cognitive performance during hypoglycaemia in people with diabetes have not shown a relationship with intelligence, or a surrogate marker such as the number of years of education [9.17.19], but only a small number of studies have examined this possibility.

3.5 Diagnosis of Diabetes

Whether diabetes *per se* influences cognitive performance during hypoglycaemia has been addressed by measuring cognitive function before, during, and after acute insulin-induced hypoglycaemia (arterialised blood glucose 1.8-2.0 mmol/l) in 10 men with type 1 diabetes and in 12 non-diabetic men, matched for age and baseline performance on a variety of cognitive tests [32]. At euglycaemia, no between-group differences in cognitive performance were apparent; during hypoglycaemia cognitive performance deteriorated significantly in both groups. However, during hypoglycaemia a greater degree of cognitive impairment occurred in those with type 1 diabetes, suggesting that diabetes confers greater susceptibility to neuroglycopenia. This could represent a “diabetic encephalopathy”, developing as a consequence either of repeated exposure to severe hypoglycaemia or from the effects of chronic hyperglycaemia [32,33]. However, cognitive function at baseline did not differ between the two groups and the diabetic subjects had higher blood glucose concentrations before the induction of

hypoglycaemia, necessitating a greater reduction in blood glucose to achieve equivalent hypoglycaemia. This may have influenced the magnitude of cognitive impairment.

In two other studies no differences between diabetic and non-diabetic subjects were noted in cognitive performance during acute hypoglycaemia [10,16], and in a further study of diabetic and non-diabetic subjects [34], differences in cognitive performance between the groups were observed at baseline, thus precluding interpretation of the effects of diabetes on responses during hypoglycaemia.

3.5.1 Other Diabetes-Related Clinical Variables

No relationship has been observed between the degree of cognitive dysfunction during hypoglycaemia and variables such as duration of diabetes [16,17,21,35], age of onset of diabetes [18,19], and the magnitude of the counterregulatory hormonal responses to hypoglycaemia [9,16]. A greater deterioration in cognitive performance during acute hypoglycaemia in people with type 1 diabetes was associated with a history of previous hypoglycaemia coma [19,20].

3.5.2 Glycaemic Control

Strict glycaemic control alters the glycaemic thresholds at which symptomatic and counterregulatory hormonal responses to hypoglycaemia are initiated, requiring lower blood glucose to trigger these responses [37,38]. However, evidence that the threshold for the cognitive dysfunction during hypoglycaemia is modified by the nature of glycaemic control is conflicting.

Cognitive function during hypoglycaemia was compared in eight subjects with type 1 diabetes with good glycaemic control (mean HbA_{1c} 8.0%) and nine subjects with poor glycaemic control (mean HbA_{1c} 11.8%) [10]. The median blood glucose for the threshold for cognitive dysfunction did not differ between the groups. Similar results were observed when the four-choice reaction time was measured during acute hypoglycaemia in eight subjects with type 1 diabetes who had good glycaemic control (mean HbA_{1c} 7.7%) and 10 who had sub-optimal control (mean HbA_{1c} 10.1%) [39]. Several other studies [10,14,31,40] have shown no correlation with quality of glycaemic control.

Measurement of event-related potentials can provide an objective quantitative assessment of cognitive function. The P300 component of these sensory evoked potentials is generated endogenously when a subject is required to discriminate and memorise a specific task-specific stimulus. Its latency reflects the speed of information processing and correlates with attention and short-term memory. Ziegler and colleagues [40] examined the effect of glycaemic control on P300 event-related potentials during hypoglycaemia. Eighteen people with type 1 diabetes were studied, seven of whom had a mean HbA_{1c} of 6.3% while 11 had a mean HbA_{1c} of 9.1%. No significant difference in P300 latency between the two groups was present at baseline. However, the glycaemic threshold at which a significant increase of P300 latency was first detected was 1.6 mmol/l in subjects with strict glycaemic control, and 3.5 mmol/l in those with poorer control. A study that examined the effect of hypoglycaemia on P300 potentials in people with intensively and conventionally treated diabetes reported similar results [41]. Thus, neurophysiological measurements suggest that glycaemic control can influence blood glucose thresholds, and may be more sensitive than cognitive tests. A significantly poorer performance was observed in an auditory reaction time test during acute

hypoglycaemia in 15 subjects with type 1 diabetes who had strict glycaemic control (mean HbA1c 6.9%) compared with subjects with less strict glycaemic control (mean HbA1c 8.8%) [13]. The subjects with strict glycaemic control had a history of more episodes of hypoglycaemic coma, which may have influenced their performance

3.6 Antecedent Hypoglycaemia

The thresholds for counterregulatory hormonal responses to hypoglycaemia are modified by preceding exposure to hypoglycaemia (antecedent hypoglycaemia), [42,43], but its impact on the glycaemic threshold for cognitive dysfunction is difficult to assess; different degrees and durations of antecedent hypoglycaemia have been studied, and the interval between the episode of antecedent hypoglycaemia and subsequent exposure to further hypoglycaemia has varied considerably between studies, so preventing comparison.

Nine non-diabetic volunteers were exposed to antecedent hypoglycaemia (arterialised blood glucose 3.2 mmol/l) or euglycaemia (blood glucose 5.2 mmol/l) for two hours, before a stepped hypoglycaemic clamp was performed 90 minutes later. Cognitive function was assessed using Logical Memory, a test of immediate verbal memory and the Digit Symbol Substitution Test, a test of general psychomotor performance [44]. During the later hypoglycaemia, equivalent deterioration of the Digit Symbol Substitution Test scores occurred irrespective of exposure to antecedent hypoglycaemia or euglycaemia, but the performance during hypoglycaemia in the logical memory task was preserved following antecedent hypoglycaemia. This suggests that an adaptive response to exposure to low blood glucose allowed the preservation of memory during subsequent hypoglycaemia.

Asymptomatic nocturnal hypoglycaemia is very common in type 1 diabetes [45,46], and its impact on cognitive function has been examined during hypoglycaemia induced the following morning. Veneman and colleagues [47] measured cognitive function during hypoglycaemia in 10 healthy volunteers after induction of asymptomatic nocturnal hypoglycaemia (plasma glucose 2.4 mmol/l) for two hours, and also after a euglycaemia study in which saline was infused overnight instead of insulin. When hypoglycaemia was induced on the following morning, cognitive dysfunction was significantly less after the antecedent nocturnal hypoglycaemia, compared to nocturnal euglycaemia. In addition, the plasma glucose concentration at which cognitive dysfunction developed was significantly lower following antecedent hypoglycaemia. Fanelli and colleagues [48] also demonstrated that a single episode of moderate (and asymptomatic) antecedent nocturnal hypoglycaemia caused less cognitive dysfunction during a subsequent episode of controlled hypoglycaemia the following day. In this study, the effect of antecedent nocturnal hypoglycaemia (arterialised plasma glucose 2.8 mmol/l) was examined in 15 people with type 1 diabetes. The subjects were exposed to either nocturnal hypoglycaemia or euglycaemia for 3.5 hours. When cognitive function was tested during hypoglycaemia induced on the following day, cognitive dysfunction was less severe after nocturnal hypoglycaemia compared with nocturnal euglycaemia. In particular, there was relative preservation in performance of tasks assessing attention and pattern recognition, but not in tasks of delayed verbal memory and information processing. These studies suggest that after *recent* antecedent hypoglycaemia the glycaemic thresholds for hypoglycaemia-induced cognitive dysfunction are shifted to lower plasma glucose concentrations.

By contrast, a study of 16 non-diabetic subjects by Hvidberg and colleagues [49] showed that the glycaemic thresholds for cognitive dysfunction during hypoglycaemia

were not altered after a period of antecedent hypoglycaemia. In the afternoon, the subjects were exposed for two hours to either moderate hypoglycaemia (mean blood glucose 2.6 mmol/l) or, on a separate occasion, to euglycaemia (4.8 mmol/L). The following morning, cognitive function tests assessing information processing, attention, pattern recognition and memory were administered during controlled hypoglycaemia. No significant effect overall of exposure to antecedent hypoglycaemia was observed on subsequent hypoglycaemia-induced cognitive dysfunction. However, when performance of individual tasks at specified blood glucose concentrations was examined, the deterioration in tasks of attention and pattern recognition was less when the plasma glucose concentration was reduced from 2.8 to 2.5 mmol/l.

No effect of antecedent hypoglycaemia on hypoglycaemia-induced cognitive dysfunction has been found in other studies. Dagogo-Jack *et al* exposed 16 people with type 1 diabetes either to antecedent hypoglycaemia or to euglycaemia induced during the afternoon [50]. Cognitive function during hypoglycaemia induced on the following morning was not affected significantly by the antecedent hypoglycaemia. Indeed, deterioration in some aspects of cognition such as attention was greater following antecedent hypoglycaemia. In another study [51], eight people with type 1 diabetes were exposed to antecedent hypoglycaemia (arterialised blood glucose 2.8 mmol/l) or euglycaemia (blood glucose 5.0 mmol/l), which was followed, after an interval of two days, by a further episode of hypoglycaemia. The four-choice reaction time test was used to examine cognitive function, and the decrement in performance in response to hypoglycaemia was unaffected by exposure to antecedent hypoglycaemia, despite blunting of the noradrenaline response to antecedent hypoglycaemia. Other studies [52,53] reported that antecedent nocturnal hypoglycaemia had no effect on cognitive performance the following day.

Finally, a study by Ovalle and colleagues [54] investigated the impact of recurrent antecedent hypoglycaemia on cognitive performance during subsequent stepped hypoglycaemia. Six patients with type 1 diabetes were exposed either to two hours of recurrent hypoglycaemia (blood glucose 2.8 mmol/l) or to two hours of hyperglycaemia (8.3 mmol/l) twice weekly, for a period of one month. Following recurrent antecedent hypoglycaemia, cognitive function during hypoglycaemia was significantly less impaired. Some evidence exists that the glycaemic thresholds for the impairment of cognitive function may be shifted to commence at lower blood glucose concentrations in people who are exposed to recurrent hypoglycaemia, but definitive studies are awaited.

3.7 Duration of hypoglycaemia: (short-term cerebral adaptation)

The brain appears to have an inherent ability to adapt to repeated exposure to low blood glucose and function normally although the supply of glucose is limited. This is apparent by the shift of the glycaemic thresholds for counterregulatory hormonal secretion and symptom generation to lower blood glucose levels. The glycaemic threshold for the onset of cognitive dysfunction may also be modified, but this is less certain. The putative mechanism underlying cerebral adaptation is unknown but may involve up regulation of glucose transporters, which control the rate of glucose transport into neurones or an enhanced ability to use alternative fuels.

In two separate studies by Kerr and colleagues, performance in a simple reaction time test was studied during prolonged hypoglycaemia in people with [55], and without [56] type 1 diabetes. Arterialised blood glucose concentration was clamped at 3.5 mmol/l for one hour then reduced to, and maintained at, 2.8 mmol/l for 90 minutes in the people with type 1 diabetes [55] and 3.0 mmol/l for 60 minutes in the people without

diabetes [56]. Reaction times were measured at baseline, and twice at each glucose plateau. Initially these reaction times slowed when the blood glucose concentration was lowered from 3.5 mmol/l to the blood glucose nadir, compared to the baseline scores. However, after prolonged exposure to hypoglycaemia the reaction times improved towards those achieved at baseline, in parallel with a fall in symptom score. Therefore, as hypoglycaemia continued, symptomatic awareness declined and cognitive function appeared to improve, while counterregulatory hormones remained elevated. The findings of these studies [55,56] have been interpreted as demonstrating some degree of cerebral adaptation during mild hypoglycaemia. However, apart from the inadequacy of the cognitive assessments, the study design is fundamentally flawed. Many cognitive functions are not impaired until blood glucose reaches a level lower than 2.8 mmol/l, and a separate euglycaemia condition in the same subjects indicated that the reaction time changes were associated with a practice effect, so that the performance improved with increasing application. No statistical comparison of the results from the hypoglycaemia and euglycaemia clamps was provided.

By contrast, Gold and colleagues [57] found no evidence of cerebral adaptation in 24 non-diabetic subjects following exposure to 40-60 minutes of hypoglycaemia (arterialised blood glucose 2.5 mmol/l). This study was more scientifically robust in that an extensive cognitive test battery was utilised, with statistical adjustment for potential practice effects by comparing the hypoglycaemia clamp results with those obtained during a separate euglycaemic clamp. However, the duration of hypoglycaemia (60 minutes) was relatively short; a longer period of exposure to hypoglycaemia may be necessary to induce cerebral adaptation.

The effect of prolonged hypoglycaemia on cerebral adaptation was investigated by Boyle and colleagues [58] whereby a group of 12 non-diabetic volunteers were exposed to

hypoglycaemia for four days. On the first day, a stepped hypoglycaemic clamp was performed, lowering blood glucose concentration from 4.7 to 2.5 mmol/l. At each plateau of blood glucose, measurements were made of hypoglycaemia symptoms, counterregulatory hormones, cognitive function and brain glucose uptake. The arterialed blood glucose concentration was maintained at 2.9 mmol/l for 56 hours. On the final day, experimental hypoglycaemia was repeated. The glycaemic thresholds at which the subjects developed symptoms, released counterregulatory hormones and developed cognitive dysfunction had shifted to lower blood glucose levels compared to the first hypoglycaemia study. In the first study, brain glucose uptake (determined by measuring glucose concentrations in arterial and jugular venous blood, and multiplying the difference by the cerebral blood flow, estimated by a tracer method) became impaired at a blood glucose concentration of 3.6 mmol/l, but on the final day it was preserved throughout hypoglycaemia. Adaptation appears to have occurred within the brain following prolonged exposure to a low blood glucose level, so preserving brain glucose uptake and protecting cerebral function during hypoglycaemia. However results must be interpreted with caution possible practice effects of the cognitive tasks were not evaluated, and the statistical analysis compared cognitive performance at each blood glucose level within a clamp with its own baseline. Interaction analyses between the clamps were not performed, thereby increasing the risk of a spurious effect emerging in line with the experimental hypothesis.

3.8 Impaired awareness of hypoglycaemia

Assessment of the effect of impaired awareness of hypoglycaemia on cognitive function during acute hypoglycaemia is hindered by the lack of a consensus definition of this

condition. Moreover, it is difficult to separate the confounding influences of recent antecedent hypoglycaemia and strict glycaemic control, which are inter-related. The effects of insulin-induced hypoglycaemia on subjective detection of hypoglycaemia, and on cognitive function using the four-choice reaction time test, were studied in 15 people with type 1 diabetes [35]. Blood glucose was lowered to 2.5 mmol/l and maintained at this level for 30 minutes. Subjects were asked if they felt hypoglycaemic and to score symptoms. At a blood glucose of 2.5 mmol/l, only four of the 15 subjects recognised that their blood glucose was low. The subjects were not matched for IQ, and those who had impaired awareness of hypoglycaemia had a longer duration of diabetes and lower mean glycated haemoglobin than the subjects with normal awareness. Cognitive performance was affected to the same degree during hypoglycaemia in the subgroup with intact symptomatic awareness, compared to the larger number with impaired awareness. This study indicates that the glycaemic threshold for the generation of symptoms varies between individuals, and is influenced by glycaemic control and factors such as duration of type 1 diabetes, but actual impairment of hypoglycaemia awareness was not demonstrated.

Zammitt and colleagues [59] have shown that the decrement in cognitive ability during hypoglycaemia is less in people with impaired awareness compared to subjects who have normal awareness of hypoglycaemia. Twenty subjects with type 1 diabetes and normal self-assessed hypoglycaemia awareness (12 male, median HbA_{1c} 8.7%, median age 30 years) and 15 subjects with impaired hypoglycaemia awareness (6 male, median HbA_{1c} 8.2%, median age 34 years) were exposed to euglycaemia (blood glucose 4.5 mmol/l) and hypoglycaemia (2.5 mmol/l). The hypoglycaemia-aware group suffered impairment of short-term verbal memory at 2.5 mmol/l, whereas the unaware group did not. This finding is consistent with cerebral adaptation being present in the unaware group.

By contrast, in an earlier study also from our centre, Gold and colleagues [60] demonstrated that people with type 1 diabetes with impaired awareness of hypoglycaemia [61] exhibited a trend towards greater cognitive dysfunction during acute hypoglycaemia, which persisted for longer following restoration of euglycaemia, compared to subjects with normal awareness. The aware and unaware subjects in this study were matched for pre-morbid IQ, duration of diabetes, HbA1c and exposure to previous episodes of hypoglycaemia. There was, however, a difference in IQ between the two groups (0.5 standard deviation), with the unaware subjects having lower mean scores on the Alice Heim 4 task (a test of general intelligence). Although this difference was not statistically significant, it may have confounded the study results.

Symptomatic awareness of hypoglycaemia may be restored in people who have impaired awareness of hypoglycaemia if hypoglycaemia is avoided [62]. Studies examining the effect of the restoration of hypoglycaemia awareness on the glycaemic thresholds for the development of cognitive dysfunction during acute hypoglycaemia have demonstrated conflicting results. Cranston and colleagues [63] investigated the effects of avoiding hypoglycaemia in 12 men with type 1 diabetes, aged 28-55 years, who had a duration of diabetes between 11 and 32 years. All had impaired awareness of hypoglycaemia, but six had strict glycaemic control (mean HbA1c 6.5%) and six had a higher mean HbA1c (8.2%). After a period of avoidance of hypoglycaemia for three weeks (which took a mean of four months to achieve), the hormonal and symptomatic responses to controlled hypoglycaemia both increased in magnitude. However, the glycaemic threshold for cognitive dysfunction (assessed solely by the four choice reaction time test) did not change, with deterioration in cognitive function occurring at similar blood glucose concentrations (2.8 mmol/l) in all patients, before, and after, the period of hypoglycaemia avoidance. By contrast, Fanelli and colleagues [64] showed that

restoration of awareness of hypoglycaemia was associated with a change in the glycaemic threshold for cognitive dysfunction during acute hypoglycaemia in 16 people with type 1 diabetes, all of whom had impaired awareness of hypoglycaemia. Cognitive function during, and symptomatic awareness of, hypoglycaemia were measured before, and after a period of avoidance of hypoglycaemia for two weeks. Counterregulatory hormonal and symptomatic responses to hypoglycaemia improved following hypoglycaemia avoidance, and the blood glucose at which cognitive dysfunction commenced was higher after the period of avoidance of hypoglycaemia. In addition, the degree of cognitive dysfunction was less at a given glucose concentration. These changes were maintained following a year of hypoglycaemia avoidance [64], and were also observed in subjects with impaired awareness of hypoglycaemia who had type 1 diabetes of short duration [65].

Similar observations were made by Mitrakou and colleagues [66] in non-diabetic people with insulinomas who were exposed to chronic hypoglycaemia. Surgical resection of the tumour reversed impaired awareness of hypoglycaemia and deficient counterregulation, while cognitive dysfunction became more evident during hypoglycaemia.

3.9 Pharmacological agents

Structural similarities exist between the pancreatic beta cell and glucose-sensitive neurones within the hypothalamus, and sulphonylurea receptors are widely distributed in neuronal cells throughout the brain. Agents that alter membrane channels for potassium adenosine triphosphate (KATP) in pancreatic beta cells have been shown to improve cognitive function during acute neuroglycopenia. In a study of 10 non-diabetic males [67], performance of the four-choice reaction time test deteriorated at a glycaemic

threshold of 2.5 mmol/l during treatment with glibenclamide, compared to of 3.0 mmol/l with diazoxide and at 2.9 mmol/l with placebo [67].

Although the brain primarily relies on glucose under periods of prolonged fasting or during hypoglycaemia it can utilise alternative fuels which potentially could protect cognitive function during hypoglycaemia. Evans and colleagues [68], used a hyperinsulinaemic glucose clamp to lower plasma glucose to 2.5 mmol/l while infusing intravenous alanine or saline in seven non-diabetic males. Cognitive function, assessed by the Stroop colour-word test, deteriorated less during alanine compared to saline infusion, although performance in several other cognitive tests did not improve. Alanine may be utilised by the brain as a metabolic fuel during hypoglycaemia in place of glucose, or may enable an increased availability of lactate. Page and colleagues from Yale examined the effects of medium chain fatty acids, which are rapidly metabolised into ketones, in their ability to protect cognitive function during a hyperinsulinaemic glucose clamp [69]. Using a battery of tests to assess multiple cognitive domains they demonstrated that ingestion of the medium chain fatty acids lead to preservation of cognitive performance in the domains of immediate verbal memory, delayed verbal memory, verbal memory recognition, DSS and total map searching. Reassuringly the medium chain fatty acids had no effect on the adrenergic hormonal or symptomatic response to hypoglycaemia. Similar results were also observed by Rossetti after oral administration of amino acids [70].

Modafinil inhibits the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and its effects on cognitive function during acute hypoglycaemia have been studied [71]. Nine healthy male volunteers were randomly assigned to take either 200mg of modafinil or a placebo, following which euglycaemia was maintained or plasma glucose reduced to 2.4 mmol/l. Modafinil had no effect on cognitive

performance during euglycaemia. However, in the modafinil-treated group performance in two cognitive function tasks (the Stroop colour-word test and simple reaction time) deteriorated less during hypoglycaemia.

Alcohol and hypoglycaemia independently affect cognitive function adversely. Cheyne and colleagues [72] demonstrated that the additive effects of hypoglycaemia and alcohol in 17 healthy subjects who were studied during (a) euglycaemia (blood glucose 4.5 mmol/l) with placebo, (b) euglycaemia with alcohol, (c) hypoglycaemia (2.8 mmol/l) with placebo and (d) hypoglycaemia with alcohol. The blood alcohol concentration was identical both arms at 43 mg/dl (2.4mmol/l). The administration of alcohol during euglycaemia was associated with deterioration in performance in the Four-Choice Reaction Time and Trail Making B tests, while the hypoglycaemia and placebo arm was associated with deterioration in performance in the Four-Choice Reaction Time test alone. However, when alcohol was combined with hypoglycaemia the deterioration in performance in all of the cognitive function tests was significantly augmented.

Caffeine heightens the symptomatic and counterregulatory responses to hypoglycaemia, thereby increasing recognition of hypoglycaemia, and several studies have examined the potential effect of caffeine on cognitive function. One study [73] showed a very small improvement in performance in a single cognitive test (the Four-Choice Reaction Time test) during hypoglycaemia, but most studies have demonstrated no such effect [73-75].

3.10 Mood

Acute hypoglycaemia has a significant effect on mood [4,76-81]. Changes in emotions are common and may influence cognitive performance during hypoglycaemia. Moderate hypoglycaemia may induce a state of anxious tension, unhappiness and low

energy, and even irritability and anger. Considerable inter-individual differences are observed in the effects of hypoglycaemia on mood and it appears that an idiosyncratic relationship exists in individual subjects between mood and low blood glucose. This discrepant effect between individuals may have a direct influence on their cognitive performance during acute hypoglycaemia.

3.11 Conclusions

Moderators of cognitive performance during hypoglycaemia may have important influences on people with insulin-treated diabetes in relation to their performance during driving or at work, which can be affected adversely by exposure to hypoglycaemia.

Individual differences in susceptibility to the effects of acute hypoglycaemia on cognitive function may be mediated by interacting factors. Increasing age and male gender may increase susceptibility to the effects of hypoglycaemia on intellectual function and, less convincingly, the presence of diabetes *per se* and higher intelligence may confer a greater predilection for cognitive impairment in the presence of neuroglycopenia. However, in type 1 diabetes, the duration of the disorder, the age of onset and the integrity of the hormonal counterregulatory responses to hypoglycaemia do not appear to be important determinants of the nature or degree of cognitive decrements during hypoglycaemia. Furthermore, evidence exists that prolonged or repeated exposure to moderate hypoglycaemia induces cerebral adaptation as manifested by a subsequent improvement in cognitive performance or the absence of any adverse effect on intellectual abilities at the same degree of hypoglycaemia.

The impact of impaired awareness of hypoglycaemia, strict glycaemic control and antecedent hypoglycaemia on the ability of the brain to adapt to frequent exposure to neuroglycopenia remains undecided. Some studies have supported the premise that the glycaemic thresholds for the impairment of different domains of cognitive function are shifted to commence at lower blood glucose concentrations in people who are exposed to recurrent hypoglycaemia, in a manner analogous to the shift in glycaemic thresholds for counterregulatory hormonal and symptomatic responses. However, a shift in glycaemic thresholds for cognitive dysfunction has not been demonstrated consistently. After discounting investigations that have obvious methodological limitations, most studies demonstrating a shift in cognitive thresholds have utilised either a battery of cognitive function tests or neurophysiological tests that measure changes in sensory evoked potentials. By contrast, the studies that did not demonstrate any change in glycaemic thresholds have usually utilised a single or, at most, two cognitive tasks [11]. It is likely that not all cognitive tests are affected to a similar degree by conditions such as impaired awareness of hypoglycaemia, strict glycaemic control or recurrent antecedent hypoglycaemia, so that limited cognitive testing may be inadequate to demonstrate an effect on glycaemic thresholds [82].

With studies that are appropriately counterbalanced, robust effects of moderate hypoglycaemia on a number of cognitive domains can be found. However, identifying the reasons for individual differences in these effects is more difficult. The study designs are largely between-subjects as opposed to within-subjects, which have less power. Furthermore, the difference in an effect between two groups typically will be relatively small. These facts combine to make the discovery of the factors underlying differences in response to hypoglycaemia scientifically demanding.

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Chapter 4

Research Aims

4.1 Research Aims

4.1.1 Hypoglycaemia and cognitive function

As human brain is dependent on a continuous supply of glucose as its main source of energy, cerebral deprivation of glucose rapidly causes cognitive dysfunction through the direct effects of acute neuroglycopenia [1,2]. An extensive body of literature already exists on the effects of hypoglycaemia on cognitive function. The aims of the following studies were to investigate the effects of hypoglycaemia on as yet unexplored aspects of cognitive function and apply novel methods of analysis to identify in greater depth the effects of hypoglycaemia on cognitive function.

The effect of hypoglycaemia on psychomotor function, unlike other cognitive domains that have received detailed examination, has attracted very limited systematic study. Psychomotor performance encompasses motor strength, hand-eye co-ordination, balance, dexterity, tracking, and other skills. These skills are important for many common activities of daily life in which hand control and fine visuo-motor coordination play a critical role, during tasks such as driving and performance at work.

The few studies that specifically have examined the effects of hypoglycaemia on psychomotor function have been limited by an inadequate degree of hypoglycaemia or by limitations in study design. Stevens and colleagues [3] examined the effects of experimentally-induced hypoglycaemia on psychomotor function, using the glucose clamp technique, in 12 adult healthy volunteers. The psychomotor test battery consisted of two simple reaction time tests, finger tapping test (FTT), trail making, critical flicker fusion (CFF), Digit Symbol (DS) and four-choice reaction time (4CRT). At a blood glucose nadir of 3.4 mmol/L two tests deteriorated significantly: trail making ($p < 0.05$) and DSS ($p < 0.03$). The blood glucose nadir that was chosen by the investigators would

not be considered to be sufficiently low to significantly affect cognitive functions, as blood glucose needs to fall below 3.0 mmol/L before many cognitive domains are affected [4-7].

The effects of hypoglycaemia on the PSE Syndrom-Test (consisting of DSS, Digit Connection, Aiming Centre I, Aiming Centre II, [requiring the subject to mark the centre of a fixed number of circles within an allocated time] and Line Tracing Time & Errors) were studied in 10 people with type 1 diabetes in a study by Lingenfelser and colleagues [8]. During hypoglycaemia a significant decrement was observed in fine motor skill and coordination (Aiming Centre I &II, Line Tracing Error tests). However the results must be interpreted with caution, because the participants did not undergo testing in a counterbalanced fashion, but were subjected to the euglycaemia test battery followed by the hypoglycaemia test battery during the same experimental session. This experimental design meant that by practice effects and fatigue were completely confounded with the experimental manipulation (euglycaemia versus hypoglycaemia).

To perform a more comprehensive assessment of psychomotor function during hypoglycaemia it is necessary to examine aspects of sensory processing, the central integration and processing responses, overt motor responses and overall sensori-motor coordination [9], (Figure 4.1). Study participants usually tolerate controlled hypoglycaemia for a maximum of about one hour, so it is not feasible to examine all aspects of psychomotor function during one period of hypoglycaemia. Study 1 (chapter 5) therefore examines the effects of acute, moderate, insulin-induced hypoglycaemia (blood glucose 2.5 mmol/L) on psychomotor functions, utilising a glucose clamp technique in a randomised, counterbalanced design, with particular attention placed on examining overt motor responses and overall sensori-motor coordination, as these have direct relevance to everyday tasks. The study was performed in both healthy volunteers

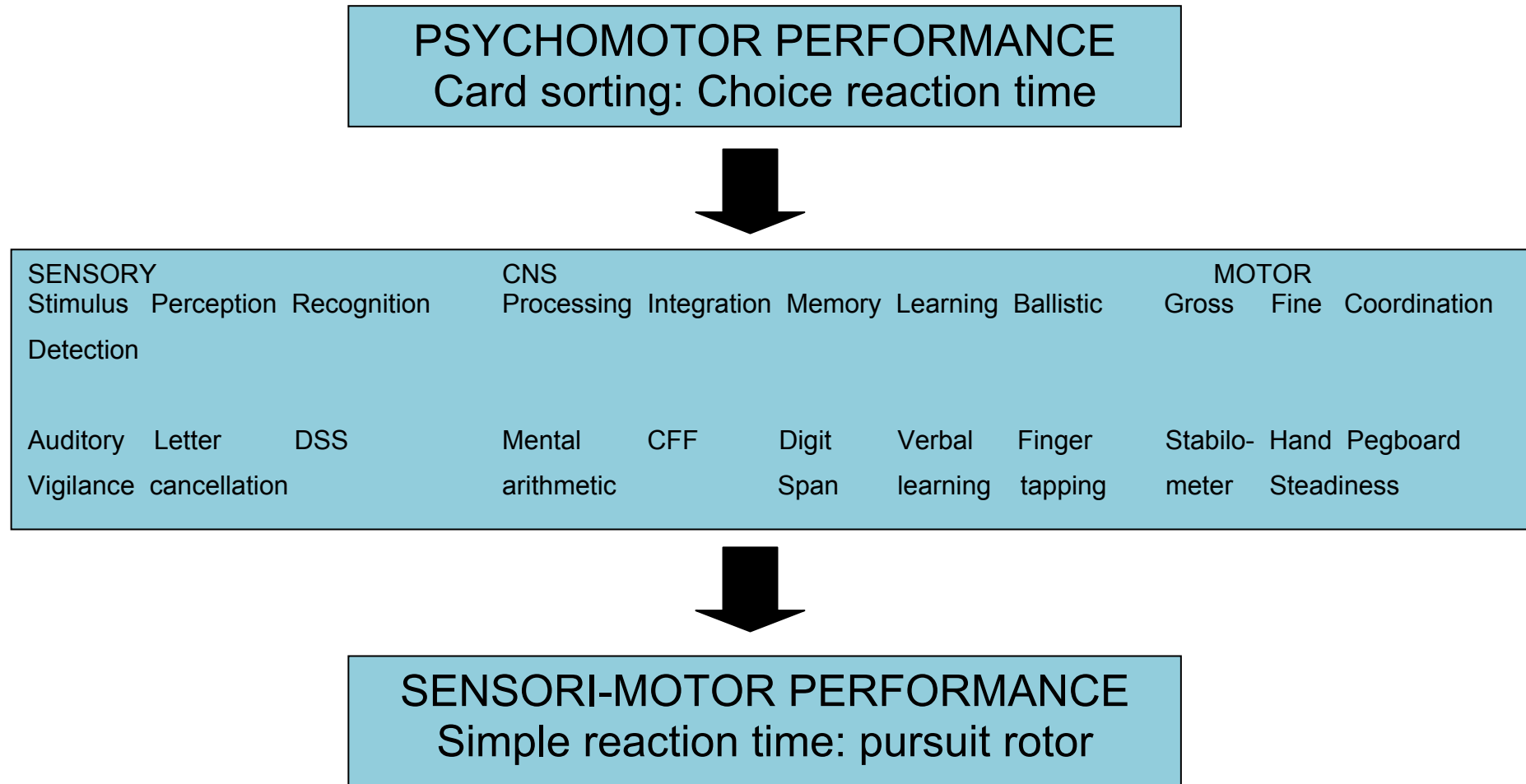
and people with type 1 diabetes to ascertain if any differences existed in the effects of hypoglycaemia on psychomotor function between the groups.

Modelling the effects of hypoglycaemia on a two-choice task in adult humans

As described above reaction time encompasses one aspect of psychomotor function. It is already known that hypoglycaemia causes reaction times to be slower and more variable [11]. In these studies, an overall test score was used, which reveals little about the precise effect of hypoglycaemia on reaction times, namely are the slowed reaction times secondary to the effects on overt motor skills, visual processing, central processing speeds etc.

Mathematical sequential modelling techniques have been developed to allow data obtained from simple reaction time tasks to provide precise quantitative predictions of the relationships between mean reaction times and the probability of correct responses and errors, and the shapes of the reaction time distribution thus allowing for dissection of the above [12,13]. Study 2 (chapter 6) therefore examines the effects of applying this mathematical sequential modelling technique to data obtained during euglycaemia and hypoglycaemia in a study of 14 healthy volunteers.

Figure 4.1: A summary of tests of psychomotor function. Adapted from [9]



4.1.2 Impaired awareness of hypoglycaemia

Despite the high prevalence of impaired awareness of hypoglycaemia in those with Type 1 Diabetes, there is currently no international consensus on its definition or the best method for identifying those suspected of having this acquired condition. This syndrome theoretically could be identified subjectively via questionnaires administered in structured clinical interviews or objectively assessing counter regulation in the course of hypoglycaemic clamps or via continuous glucose monitoring. The most common questionnaire assessment methods used in both clinical practice and in population based studies are the methods of Clarke, Gold and Pederson Bjergaard [14-16]. The methods of Clarke and Gold are relatively similar in that they ascertain whether the person with type 1 diabetes has perceived any change in symptoms to the onset of hypoglycaemia and quantify the previous exposure to severe hypoglycaemia. The method of Pederson Bjergaard simply requires the patient to respond to the question “Do you have symptoms when you have a hypo?”, requiring the selection of one response from “always”, “sometimes” or “never”. Only the patients who answer “always” are considered to have normal symptomatic awareness of hypoglycaemia, the others are designated as having impaired or absent awareness. Studies from this group have reported rates of impaired/absent awareness of up to 59% of all clinic patients [17], which are far in excess of the expected rate of impaired awareness in a standard cohort with type 1 diabetes. These rates may have occurred due to a very atypical patient population or due to the fact their simple method of identifying patients with impaired awareness of hypoglycaemia over-estimates the prevalence of this syndrome. Study 3 (chapter 7) compares the currently available methods in a randomly selected population of people with Type 1 diabetes to ascertain concordance between the methods.

The artificial nature of experimentally-induced hypoglycaemia using the glucose clamp technique, lends itself to being unsuitable for identifying impaired awareness for variety of reasons, such as the method of induction of hypoglycaemia, the supine posture, the delay in generating symptoms at different clamped blood glucose levels and so on. The ability of continuous glucose monitoring to differentiate between those with and without impaired awareness of hypoglycaemia will be examined in Study 4 (Chapter 8) with the hypothesis that those with impaired awareness of hypoglycaemia (as estimated from clinical characteristics associated with this syndrome and identification from a validated questionnaire) will have a higher rate of biochemical hypoglycaemia on CGM.

The prevalence of impaired awareness of hypoglycaemia in Type 1 Diabetes has been a neglected area of research in recent years despite major advances that have been made in managing insulin-treated diabetes. These include the introduction of insulin analogues and CSII, the intensification of insulin regimens and innovative methods of patient education (such as DAFNE) which have been anticipated to have reduced overall exposure to hypoglycaemia, which we hypothesis should have led to a decline in the prevalence of IAH. Study 5 (Chapter 9) examines the current prevalence of IAH in a large scale clinic based study.

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Chapter 5

Effects of acute insulin-induced hypoglycaemia on psychomotor function

5.1 Introduction

The effect of hypoglycaemia in causing cognitive dysfunction in general is well recognised. Recent studies have focused on how hypoglycaemia affects specific cognitive domains and the clinical relevance of any cognitive decrement that occurs [1-5].

Psychomotor function is an important domain of mental function that has not received systematic study with respect to the effect of hypoglycaemia. Psychomotor performance encompasses motor strength, hand-eye co-ordination, balance, dexterity, tracking, and other skills. Clearly, this range of motor and more psychomotor capabilities is important in many common everyday activities. Hand control, strength, and broad and fine visuo-motor coordination play a vital role in driving, work performance and domestic life. During episodes of iatrogenic hypoglycaemia, people with insulin-treated diabetes frequently have reported a deterioration of fine motor skills [6].

Previous studies of cognitive function during hypoglycaemia have occasionally included isolated psychomotor tests, but none has explored effects on this domain of psychological function comprehensively [7, 8, 9]. However, the few studies as discussed in Chapter 4 that have examined specifically the effects of hypoglycaemia on psychomotor function have been limited by a blood glucose nadir that would not be considered sufficiently low to have a significant effect on cognitive functions [10]. In other studies, a euglycaemia control arm was not incorporated in the study design, resulting in the effects of practice and fatigue being confounded by the experimental manipulation (euglycaemia versus hypoglycaemia) [11]. These problems were circumvented by the design of the present study, in which several forms of

psychomotor function were measured during hypoglycaemia both in healthy non-diabetic volunteers and in healthy adults with type 1 diabetes.

5.2 Methods

The study protocol was approved by the Lothian medical research ethics committee, and all subjects gave informed consent for participation.

5.2.1 Subjects

Twenty (11 female) non-diabetic adults, (median (IQR) age 32 (27-35) years), and sixteen (8 female) adults with type 1 diabetes were studied. The subjects with type 1 diabetes had a median (inter-quartile range) age of 40 (36-42.8) years, duration of diabetes of 15 (6-25) years and mean (SD) HbA_{1c} of 8.2 (0.6) %. No significant difference in age between the two groups (healthy non-diabetic volunteers or those with type 1 diabetes) was observed. HbA_{1c} was measured by ion exchange high performance liquid chromatography via the Bio-Rad Variant II Haemoglobin testing system (non-diabetic reference range 5.0-6.05%) and was DCCT-aligned. None of the non-diabetic group had any previous medical history or a family history of diabetes, and none was taking regular medication (other than the oral contraceptive pill). As microvascular complications have been linked to a decrease in cognitive performance the subjects with type 1 diabetes had to have no evidence of microvascular complications, including diabetic retinopathy, peripheral neuropathy or nephropathy, the latter being defined by having a urine albumin: creatinine ratio persistently above the local reference range or a serum creatinine greater than 150 $\mu\text{mol/L}$. This, however, may not be representative of all subjects with a median duration of diabetes of 15 years. None of the diabetic

participants had impaired awareness of hypoglycaemia (IAH) as assessed by a validated method [12].

5.2.2 Glucose clamp procedure

Each subject participated in two laboratory sessions, each separated by at least two weeks. The studies were conducted in the Clinical Research Facility of the Royal Infirmary of Edinburgh. During the experimental visits, subjects underwent a modified hyperinsulinaemic glucose clamp [13]. The subjects completed both experimental conditions in a counterbalanced fashion, i.e. half of the subjects underwent the euglycaemia condition first followed by the hypoglycaemia condition and the other half underwent the experimental conditions in reverse order. The subjects were not informed which condition was being studied at each visit.

Each session commenced at 8.00h following a 10 hour overnight fast. The subjects with type 1 diabetes administered their usual dose of insulin during the preceding evening, but no subcutaneous insulin was injected on the morning of the session. Studies were postponed if any of the subjects with type 1 diabetes developed either symptomatic or biochemical hypoglycaemia (blood glucose <4.0 mmol/L) during the 48 hours before each study. This resulted in one postponement. A Teflon cannula was inserted into the ante-cubital vein under local anaesthetic (2% lignocaine). This cannula was used to infuse human soluble insulin (Actrapid, Novo Nordisk pharmaceuticals, Crawley, UK) and 20% dextrose. A second cannula was inserted in a retrograde direction into a vein on the dorsum of the hand, which was placed in a heated blanket to arterialise the venous blood. Arterialised blood samples were obtained throughout the study for the measurement of whole blood glucose at the bedside using a glucose oxidase method

(Yellow Springs Instrument 2300 Stat, Yellow Springs, OH, USA). A modified hyperinsulinaemic glucose clamp technique (as described above) was used to maintain the blood glucose at predetermined levels. After a brief priming regimen, insulin was infused at a steady rate (based on whole body surface area) of $60 \text{ mU m}^{-2} \text{ min}^{-1}$ using an IVAC Signature Gold pump (Alaris Medical Systems, San Diego, CA); 20% dextrose was infused, also using a IVAC Signature Gold pump, at a variable rate depending on the blood glucose value. Arterialised blood glucose was measured initially at every 3 min, until a stable level had been achieved, and then at 5-minute intervals. At each laboratory session, arterialised blood glucose was initially stabilised at 4.5 mmol/L for a period of 30 minutes. Following this, the blood glucose concentration was either maintained at 4.5 mmol/L (euglycaemia) or lowered to 2.5 mmol/L (hypoglycaemia) and the neuropsychological tests administered. The subjects were not informed about their blood glucose concentration during any phase of the study. A period of 20 minutes was allowed to elapse between the baseline and the attainment of euglycaemia or hypoglycaemia to allow the blood glucose concentration to stabilise. The target glucose concentration was maintained for a further 10 minutes before the tests were administered and was maintain for a further 60 minutes while the tests were administered. At the end of the hypoglycaemia session the blood glucose was restored to 4.5 mmol/L. Subjects were provided with a meal after completion of each condition. Epinephrine (adrenaline) and norepinephrine (noradrenaline) concentrations were also measured at baseline, 45 minutes into the experimental condition and at the completion of each session.

5.2.3 Psychomotor tests

4 Choice Reaction Time test – Reaction time is measured using a portable device, incorporating a high contrast LCD display screen at the top with response keys arranged below in a shallow arc (numbered 1, 2, 0, 3, 4) [14]. For four-choice reaction time, the subjects have to press the corresponding key when the one of the four digits (1, 2, 3 or 4) appears on the screen. Mean and the standard deviation of the reaction times to correct trials (40 test trials, preceded by 8 practice trials) were recorded.

Grooved Pegboard – The pegboard is a test of finger and hand dexterity, and is part of the Wisconsin Neuropsychological Test Battery [15]. The subject is presented with a small board consisting of a 5x5 set of slotted holes angled in different directions, which they complete with their dominant hand. Each peg has a ridge along one side requiring it to be rotated into position. The score is the time taken to complete the task.

Tracing Test – This test assesses visuo-motor spatial ability [16]. Subjects are requested to draw a line using a digital pen (Anoto system) between two narrow parallel lines while avoiding random circles within the two lines. Accuracy is measured as the total number of times the line is crossed or a circle transected. Two scores are obtained, one for speed of completion in time and one for accuracy (errors).

Pursuit Rotor (Lafayette Instruments, IN, USA) – This test examines hand-eye coordination and fine motor control. A light target rotates around a track (at a rotation speed of 30 rpm) and the subject is required to hold a stylus over the target to track its

movement. Performance is measured by the amount of time the subject can keep the stylus on the target (time on target) during one minute.

Hand Steadiness (Lafayette Instruments, IN, USA) - The subject is required to place a metal tipped stylus in 9 progressively smaller holes without touching the sides. The stylus is held in position in each of the holes for 10 seconds. The impulse counter silently records activation if contact is made. The score is the total number of times the subject has touched the side of the holes for all nine holes.

Static Balance – This was measured by static posturography via a force platform, designed to measure the position and the magnitude of the total vertical component of force applied to it [17]. Subjects were tested on a firm surface with their eyes open. During the period of measurement (one minute) the movement of the subject's centre of mass was displayed on a computer screen and the corresponding total path length moved by the centre of mass was calculated.

Hand grip (Lafayette Instruments, IN, USA) – A hand dynamometer was placed in the subject's dominant hand at their side away from their body. The subject was instructed to squeeze the dynamometer as hard as possible, with no time constraint taking to reach the maximum grip. The score is the amount (in kg) registered at each of the three trials, with the median calculated for that hand.

5.2.4 Other tests

These were performed as a validation check, to allow comparison of the effects of hypoglycaemia as had been demonstrated in previous studies in our laboratory [2,3,8].

Digit Symbol Test (DST).

This test assesses sustained attention, speed of response and visual scanning [18].

5.2.5 Symptom scores

The subjects scored the presence and intensity of symptoms of hypoglycaemia using the Edinburgh Hypoglycaemia Symptom Scale [19]. Symptoms of hypoglycaemia are classified as *autonomic* (hunger, palpitations, sweating, tremor), *neuroglycopenic* (confusion, drowsiness, difficulty concentrating, weakness) and *malaise* (nausea, headache). Subjects scored their symptoms of hypoglycaemia at baseline and at 45 minutes into the experimental period.

5.2.6 Statistical analysis

A general linear model (repeated measures analysis of variance [ANOVA]) was used, with order of session (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia) as a between-subjects factor, and condition (euglycaemia or hypoglycaemia) as a within-subject factor (repeated measure). In the full model, including the non-diabetic volunteers and adults with type 1 diabetes, subject group and order of session were between-subjects factor with condition as a within-subjects factor. A p value of less than or equal to 0.05 was considered to be significant. Effect sizes were calculated using eta

squared (η^2). The principal measures of interest were the eta squared representing the proportion of the variance in the test scores accounted for by study condition (euglycaemia vs. hypoglycaemia). All analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

5.3 Results

5.3.1 Blood glucose

The mean (SD) fasting arterialised blood glucose in the group with type 1 diabetes was 7.1 (1.1) mmol/L on the morning of the studies. In the non-diabetic group during euglycaemia the mean blood glucose concentration was 4.45 mmol/L (0.12) and during the hypoglycaemic condition, the mean (SD) blood glucose was 2.59 mmol/L (0.11), (Figure 5.1). In the subjects with type 1 diabetes during euglycaemia the mean blood glucose concentration was 4.47 mmol/L (0.10) and during the hypoglycaemic condition, the mean (SD) blood glucose was 2.58 mmol/L (0.11), (Figure 5.2).

5.3.2 Symptoms

The scores from the hypoglycaemia symptom questionnaire were significantly higher during hypoglycaemia in both groups for autonomic ($p < 0.001$), neuroglycopenic ($p < 0.001$) and malaise symptoms ($p < 0.001$), compared to the scores obtained during euglycaemia.

5.3.3 Catecholamine concentrations

Plasma concentrations of both epinephrine ($p = 0.04$), (Figure 5.3), and of norepinephrine ($p = 0.006$), (Figure 5.4), were significantly higher during hypoglycaemia ($t = 45$ minutes) in the healthy volunteer group compared to the people with type 1 diabetes. Similar results were found in the hypoglycaemia recovery period (Figure 5.3), but all other time points revealed no difference in the concentration of catecholamines between the groups. Correlations between plasma concentrations of epinephrine and hand steadiness were non-significant for both the healthy volunteer ($r_s = 0.46$, $p = 0.06$) and the type 1 diabetes group ($r_s = 0.008$, $p = 0.84$).

Figure 5.1: Mean blood glucose concentrations (SD) during baseline and the study conditions of both the euglycaemic (●) and hypoglycaemic (▲) glucose clamps in the non-diabetic subjects.

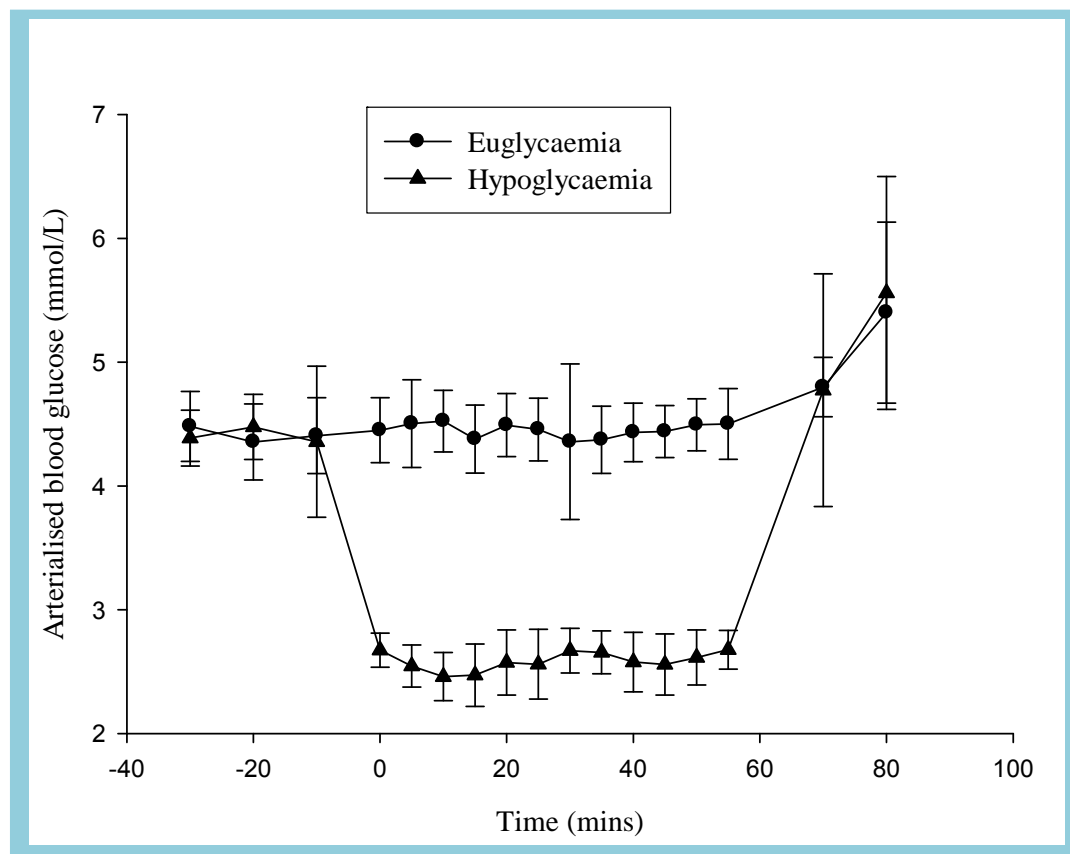


Figure 5.2: Mean blood glucose concentrations (SD) during baseline and the study conditions of both the euglycaemic (●) and hypoglycaemic (▲) glucose clamps in the subjects with type 1 diabetes.

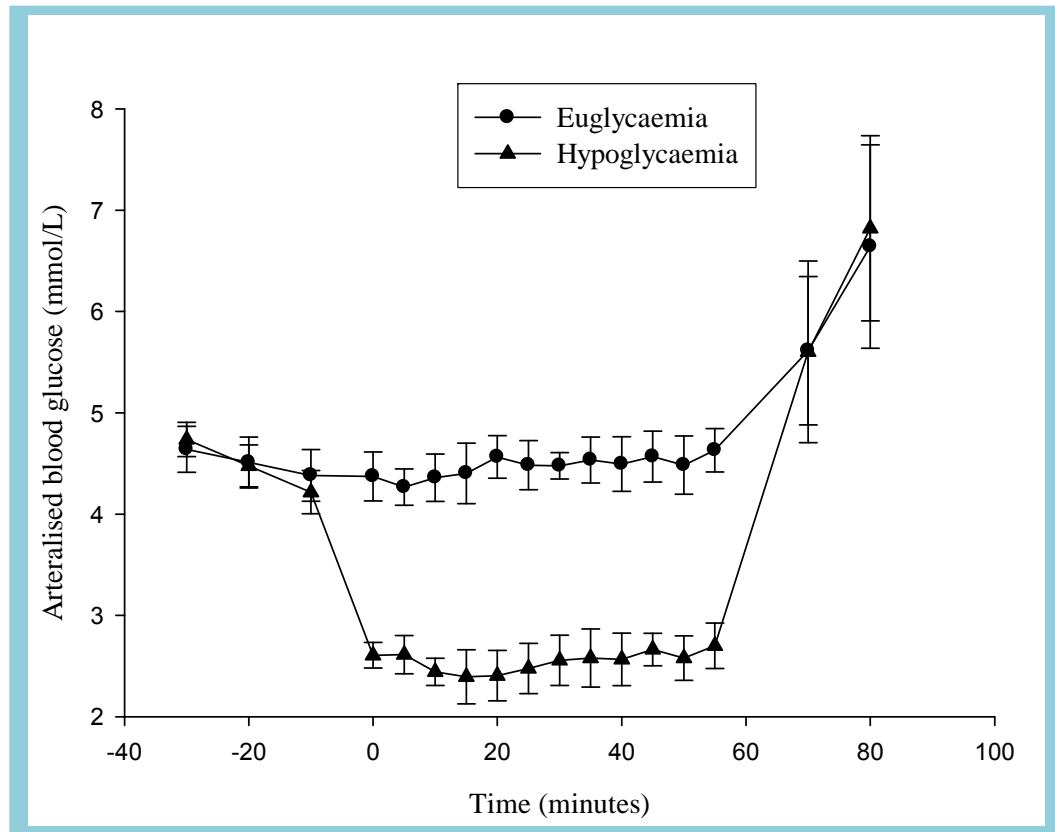


Figure 5.3: Mean plasma epinephrine (adrenaline) concentrations (SD) during study condition and recovery in both the healthy volunteers and people with type 1 diabetes.

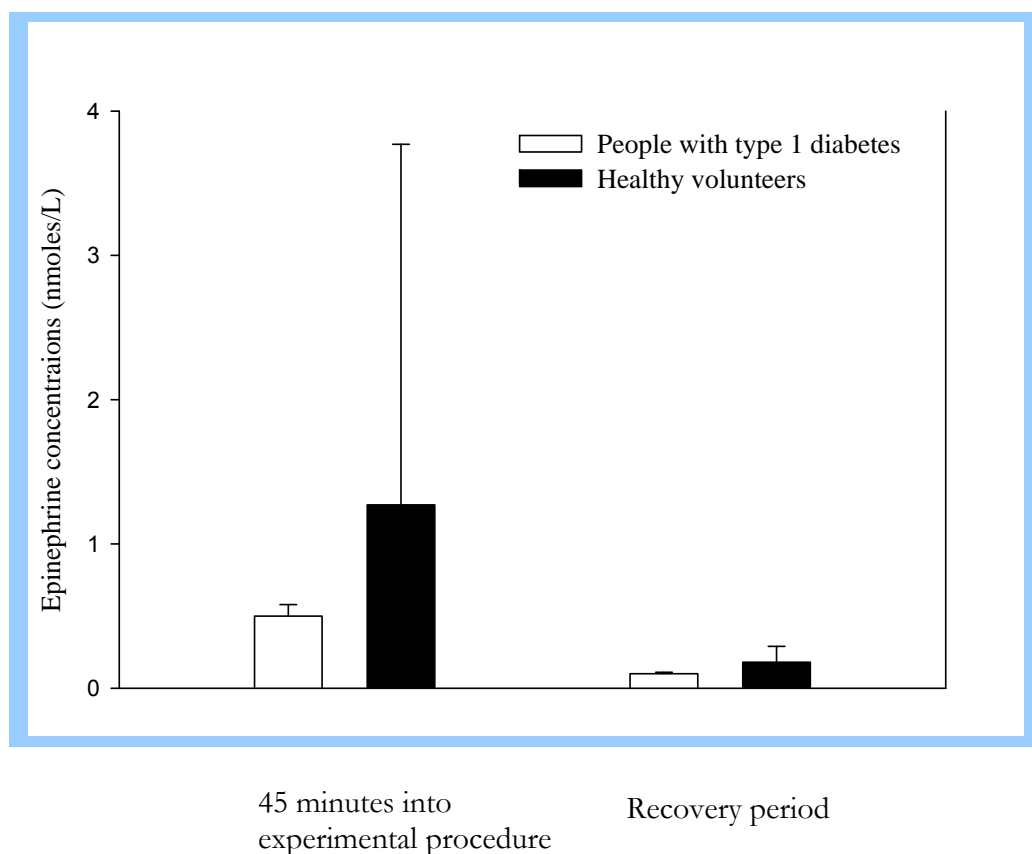
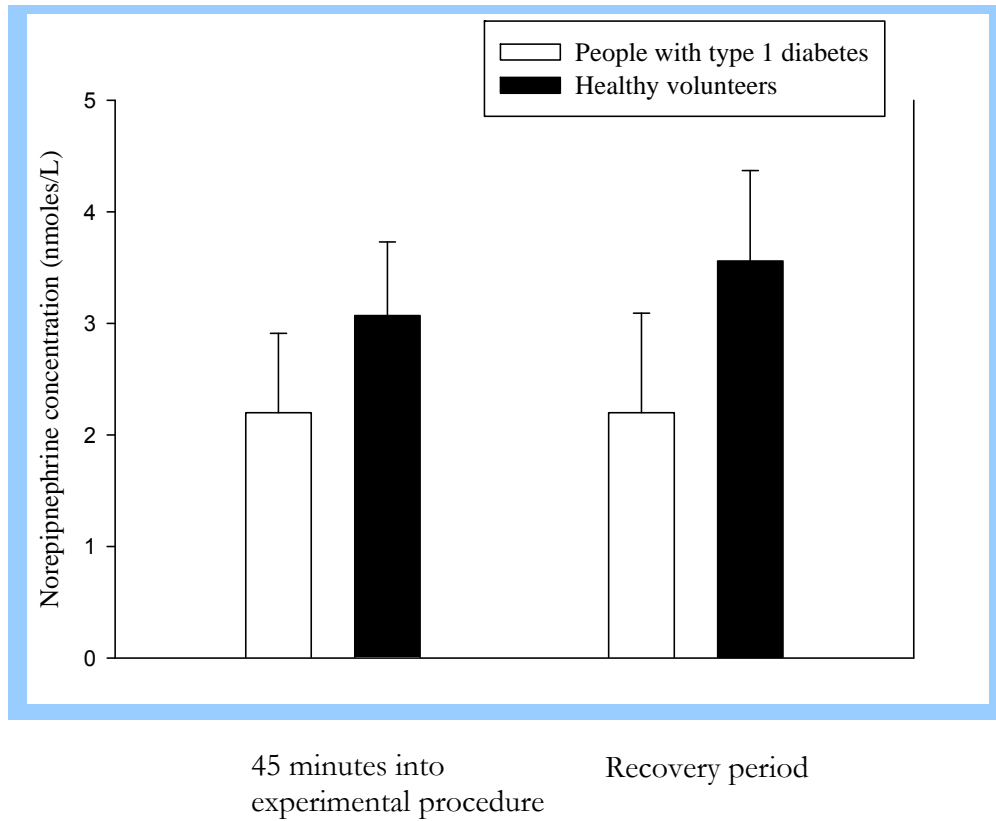


Figure 5.4: Mean plasma norepinephrine (noradrenaline) concentrations (SD) during study condition and recovery in both the healthy volunteers and people with type 1 diabetes.



5.3.4 General cognitive function tasks

The mean (SD) score of the Digit Symbol test deteriorated from 92.7 (12.6) during euglycaemia to 82.5 (9.7) during hypoglycaemia, $p < 0.001$, $\eta^2 = 0.624$ in the non-diabetic group and from 81.4 (22.6) during euglycaemia to 73.3 (21.6) during hypoglycaemia, $p < 0.007$, $\eta^2 = 0.445$ in the group with type 1 diabetes. These findings confirm that the hypoglycaemic intervention had the expected effects comparable to previous studies of similar design [2, 3, 8].

5.3.5 Psychomotor tasks

The results of mean (standard deviation) and eta squared of the psychomotor function test scores for the healthy volunteers (HV) and the people with type 1 diabetes (T1DM) during euglycaemia and hypoglycaemia (glycaemic status) are shown in table 5.1. The interaction between glycaemic status and group of subject (healthy volunteer or person with type 1 diabetes), was then also examined (Table 5.1)

Statistical analysis confirmed that no significant order effects had occurred for any of the outcome variables of the study.

Four choice reaction time

Acute hypoglycaemia caused a significant increase in mean four choice reaction times, both in the non-diabetic group ($p = 0.008$, $\eta^2 = 0.36$) and in the group with type 1 diabetes ($p = 0.02$, $\eta^2 = 0.34$). The interaction between glycaemic state and subject group, (hereafter termed the glycaemia-group interaction) was not significant ($p = 0.76$).

Grooved pegboard

In the test of hand dexterity, acute hypoglycaemia caused a significant increase in the time taken to complete the task in the non-diabetic group ($p = 0.004$, $\eta^2 = 0.37$). In the group with type 1 diabetes no significant differences were observed between the two study conditions. ($p = 0.44$, $\eta^2 = 0.045$). The interaction between glycaemic state and subject group, however was not significant ($p = 0.38$).

Hand Steadiness

The hand steadiness test demonstrated a significant decrement during hypoglycaemia ($p = 0.003$, $\eta^2 = 0.40$) in the non-diabetic group. No significant decrement in hand steadiness during hypoglycaemia was demonstrable in the group with type 1 diabetes ($p = 0.11$, $\eta^2 = 0.18$). The glycaemia-group interaction achieved statistical significance suggesting that the effects of hypoglycaemia differed significantly between the groups ($p = 0.021$).

Tracing time

No significant change in performance was observed in tracing time, between the two study conditions, either in the non-diabetic group ($p = 0.480$, $\eta^2 = 0.03$) or in the group with type 1 diabetes ($p = 0.39$, $\eta^2 = 0.06$). Non-significant differences, were also noted with tracing time errors, in subjects with ($p = 0.50$, $\eta^2 = 0.03$), and without type 1 diabetes ($p = 0.436$, $\eta^2 = 0.03$). The interaction between glycaemic state and subject group, was not significant for both tracing time ($p = 0.62$) and tracing time errors ($p = 0.70$).

Pursuit Rotor

Mean scores for time on target were significantly greater during euglycaemia compared to hypoglycaemia in the non-diabetic group ($p = 0.018$, $\eta^2 = 0.29$) and in those with type 1 diabetes ($p = 0.04$, $\eta^2 = 0.27$). The interaction between glycaemic state and subject group, was not significant ($p = 0.59$).

Static Balance

In the non-diabetic group, total body sway, assessed by static posturography, increased during hypoglycaemia under the condition of eyes open, ($p = 0.004$, $\eta^2 = 0.41$). No significant change in total body sway, was observed in the group with type 1 diabetes, under the conditions of eyes open, ($p = 0.34$, $\eta^2 = 0.08$). The glycaemia-group interaction achieved statistical significance suggesting that the effects of hypoglycaemia differed significantly between the groups ($p = 0.042$).

Hand grip

No significant deterioration of grip strength was observed during hypoglycaemia compared to euglycaemia ($p = 0.90$, $\eta^2 = 0.001$) both in the non-diabetic group and in the group with type 1 diabetes ($p = 0.96$, $\eta^2 = 0.000$). The interaction between glycaemic state and subject group, was not significant ($p = 0.90$).

Table 5.1: Results (mean and (SD)) of tests of psychomotor function during euglycaemia (Eu) and hypoglycaemia (Hypo) in 20 non-diabetic volunteers and 16 people with type 1 diabetes.

	NON-DIABETIC SUBJECTS				SUBJECTS WITH TYPE 1 DIABETES					
	Eu	Hypo	p	η^2	Eu	Hypo	p	η^2	Glycaemia –group interaction	
									p	η^2
4CRT (milliseconds)	576 (67)	616 (52)	0.008	.364	644 (10.)	687 (13)	0.023	0.340	0.762	0.003
Grooved pegboard	63.5 (9.0)	70.8 (8.6)	0.004	.371	69.7 (13.5)	72.2 (12.4)	0.443	0.045	0.376	0.025
Hand-grip (kilograms)	36.6 (8.9)	36.5 (9.3)	0.897	.001	40.8 (11.5)	39.4 (11.4)	0.961	0.000	0.902	0.000
Hand Steadiness	91.8 (52.3)	179.4 (115.7)	0.003	.404	108.5 (39.4)	125.3 (22.8)	0.111	0.183	0.021	0.159
Tracing time (seconds)	64.1 (15.1)	62.1 (11.7)	0.480	.030	62.0 (12.2)	61.7 (13.6)	0.391	0.057	0.615	0.009
Tracing time errors	11.3 (9.5)	12.6 (8.6)	0.436	.034	16.6 (8.4)	19.1 (21.0)	0.504	0.035	0.702	0.005
Pursuit Rotor (seconds)	25.1 (6.8)	21.8 (9.6)	0.018	.288	27.5 (10.5)	21.1 (10.2)	0.045	0.274	0.588	0.010
Static balance with eyes open (meters)	0.34 (0.12)	0.44 (0.17)	0.004	.414	0.39 (0.09)	0.40 (0.08)	0.337	0.077	0.042	0.184

5.4 Discussion

5.4.1 Psychomotor function

In the present study hypoglycaemia appeared to exert a differing effect between the groups with regard to certain psychomotor tests (static balance and hand steadiness). The deterioration in the performance in DST in both groups during hypoglycaemia confirmed that the blood glucose was sufficiently low to impair cognitive function, and all subjects experienced symptoms of hypoglycaemia during the study.

However in both groups, some cognitive tests were not affected by the moderate degree of hypoglycaemia, which included the line tracing and hand-grip tests. The hand-grip strength test is a simple, non-invasive measure of upper extremity muscle strength. The lack of an effect of hypoglycaemia on hand-grip function is consistent with a previous study which demonstrated that hypoglycaemia had no effect on the peripheral nervous system [18]. Tracing time was also not affected by moderate hypoglycaemia. This test of spatial ability, which has previously been demonstrated to be affected by moderate hypoglycaemia [20], is clearly too simplistic to assess this domain adequately.

Scores on only two tests, the 4CRT and pursuit rotor, were significantly affected by hypoglycaemia in both groups. The effect sizes obtained for the euglycaemia-hypoglycaemia comparison in the two studies showed almost identical effect sizes: for 4CRT, $\eta^2 = 0.36$ in non-diabetic volunteers compared with $\eta^2 = 0.34$ in subjects with type 1 diabetes; and for pursuit rotor, $\eta^2 = 0.29$ in non-diabetic volunteers vs. $\eta^2 = 0.27$ in subjects with type 1 diabetes. Previous research has demonstrated that recurrent exposure to hypoglycaemia can lead to preservation of cognitive function with regard to certain cognitive domains [21]. CRT, however does not appear to adapt, and if

hypoglycaemia is induced slowly, it deteriorates at similar levels of blood glucose in most groups of subjects, irrespective of their previous glycaemic experience or state of hypoglycaemic awareness [22]. Given that no difference was observed in pursuit rotor results between the groups we would postulate that pursuit rotor also appears not to adapt to recurrent hypoglycaemia.

The remainder of the tests namely hand steadiness, total body sway and grooved pegboard, revealed disparate results between the groups. All of the tests were significantly affected by hypoglycaemia in the non-diabetic group, with none being significantly affected in the group with type 1 diabetes. These findings appear to suggest that individuals with type 1 diabetes are less affected by hypoglycaemia than the healthy volunteers. However, a conclusion that the effects of hypoglycaemia are significantly different between the two groups requires more than simply demonstrating that the effects are significant in one group and not the other. When rigorous statistical methodology is applied to test for any differences between the groups, a significant interaction between group status and glycaemic condition would have to be observed. This was significant for two out of the three tests: hand steadiness and total body co-ordination (sway). This study therefore provides further evidence for a significant difference being exerted by hypoglycaemia when non-diabetic volunteers are compared with subjects who have type 1 diabetes. The mechanism behind this differing effect of hypoglycaemia on psychomotor function remains unknown, however may relate to the difference in sympatho-adrenal activation between the groups, a behavioural advantage in those with T1DM over the non-diabetic group derived from their previous exposure to hypoglycaemia or possibly through hypoglycaemia-induced cerebral adaptation.

Hypoglycaemia is known to result in the activation of the autonomic nervous system with subsequent release of counterregulatory hormones. Recurrent exposure to hypoglycaemia (as would be expected in the group with type 1 diabetes) attenuates this response. The endogenous effects of autonomic activation that are manifested by sweating, shaking and a pounding heart, could therefore potentially interfere with psychomotor function such as hand-eye co-ordination and fine motor control. Therefore it is possible that the differences observed in the scores obtained in the pursuit rotor test and for hand steadiness may reflect the differing catecholamine responses to hypoglycaemia that were observed between the groups. However, the autonomic features result from central autonomic neural activation (via hypothalamic centres) [23] and, whereas the rise in plasma catecholamines augments the intensity of some autonomic manifestations such as a pounding heart and tremor, it is not the principal mediator. No significant correlations were demonstrable in the present study between the plasma concentrations of catecholamines and a test of fine motor control (hand-steadiness). However, the effect sizes were modest, and a larger study would be required to investigate this possibility further. Inevitably, the present study, though very powerful for the principal within-subjects analyses, provided less statistical power for correlation analyses.

In non-diabetic volunteers and in people with type 1 diabetes, antecedent hypoglycaemia can alter the glycaemic thresholds for symptomatic and counterregulatory hormonal responses, re-setting these at lower blood glucose levels. In a small study of adults with type 1 diabetes ($n=6$), twice-weekly episodes of experimentally-induced hypoglycaemia over one month resulted in preservation of cognitive function (pattern recognition, memory, attention and information processing) [21]. While animal experiments have suggested that improved glucose uptake via glucose

transporters may contribute to the cerebral adaptation associated with recurrent hypoglycaemia [24] in humans it is not known whether regional brain differences in these processes might underlie differential adaptation of neurocognitive brain functions to recurrent hypoglycaemia [24].

Another potential explanation could be that in the group with type 1 diabetes, previous experience of coping with the effects of hypoglycaemia may confer a behavioural advantage over the non-diabetic group. Direct observation of the subjects with type 1 diabetes during administration of the cognitive function tests, gave the impression that they were concentrating on the tasks during hypoglycaemia with greater intensity than the hypoglycaemia-naïve, non-diabetic subjects. A previous clamp study by our group of 16 adults with type 1 diabetes who had normal awareness of hypoglycaemia found no difference between the subjects in their perception of their ability to concentrate during the conditions of hypoglycaemia (2.5mmol/L) and euglycaemia (4.5mmol/L) [25]. Whether a difference in ability to concentrate during hypoglycaemia occurs between people with type 1 diabetes and hypoglycaemia-naïve, non-diabetic subjects remains to be explored.

Subjects of both sexes participated in the present study. Gender differences have been observed previously in the counterregulatory hormonal responses to hypoglycaemia [26-30], but the symptomatic responses do not differ [31]. The evidence for a gender difference in degree of cognitive impairment is equivocal. In one study of non-diabetic young adults, cognitive impairment was less in women than in men in tests of selective and sustained attention and mental flexibility [32], while in a different study, this cognitive advantage in women was observed only at very mild hypoglycaemia, with no difference when blood glucose was lowered to 2.6 mmol/l [33]. While we cannot

exclude a gender difference in the effect of hypoglycaemia on psychomotor function, this is unlikely to be substantial at the degree of hypoglycaemia tested.

Caution should however be exercised before concluding that the effects of hypoglycaemia differed significantly between the two groups. While the size of the groups in the present study was well powered to examine the effect of hypoglycaemia within these groups, statistical power was insufficient for formal between-group comparisons. Given the effect sizes observed, to have high power to detect a medium effect this would require more than 60 subjects in each group, which would be very demanding to accommodate when performing studies of this nature. However, despite these reservations, the subjects with type 1 diabetes who had normal awareness of hypoglycaemia, appeared to be relatively resistant to the effects of hypoglycaemia on many aspects of psychomotor function.

5.4.2 Conclusions

In conclusion, the present study has demonstrated that moderate acute insulin-induced hypoglycaemia exerted a differing effect between the groups to certain tests of psychomotor function. The difference in response to hypoglycaemia between the healthy volunteers and people with type 1 diabetes was in some part unexpected and thus warrants further investigation utilising PET or functional magnetic resonance imaging to demonstrate differences in activation/de-activation levels during hypoglycaemia.

5.5 References

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Chapter 6

Modelling the Effects of Hypoglycaemia on a Two-Choice Task in Adult Humans

6.1 Introduction

The glucose clamp technique [1] has been used to study the effects of hypoglycaemia, typically compared with a counterbalanced euglycaemic state, on a range of cognitive and motor functions. These range from practical tasks to more information processing oriented measures. Hypoglycaemia causes deterioration in aspects of driving [2], which is of interest for reasons of health and safety and for legal reasons. Such high-level performance says little that is specific about the more precise brain functions that are affected. At a more basic level hypoglycaemia is known to cause deterioration in psychometric and neuropsychological tests assessing the cognitive domains of memory [3-5], attention [6, 7], reasoning [7], and psychomotor function [8]. At an even lower level, hypoglycaemia causes reaction times to be slower and more variable [9,10], and visual [11] and auditory [12] information processing becomes less efficient. The latter studies used tasks from psychophysics and experimental psychology. Even in these studies, an overall test score was used, not precise parameters related to cognitive processing stages. To date, there have been no studies that have examined the effects of hypoglycaemia on a task that assesses basic parameters of decision-making. Such fundamental information about the effect of fuel deprivation on the brain's basic capabilities would be useful both for basic brain science and for applied research. Here, for the first time, we examine the effects of hypoglycaemia on a task which has validated information processing parameters. The task itself is a numerosity discrimination task in which a 10x10 array is presented on a computer screen with between 31 and 70 asterisks placed randomly. The subject is required to decide whether the number of asterisks is large (greater than 50) or small (less than 50).

6.1.2 The diffusion model

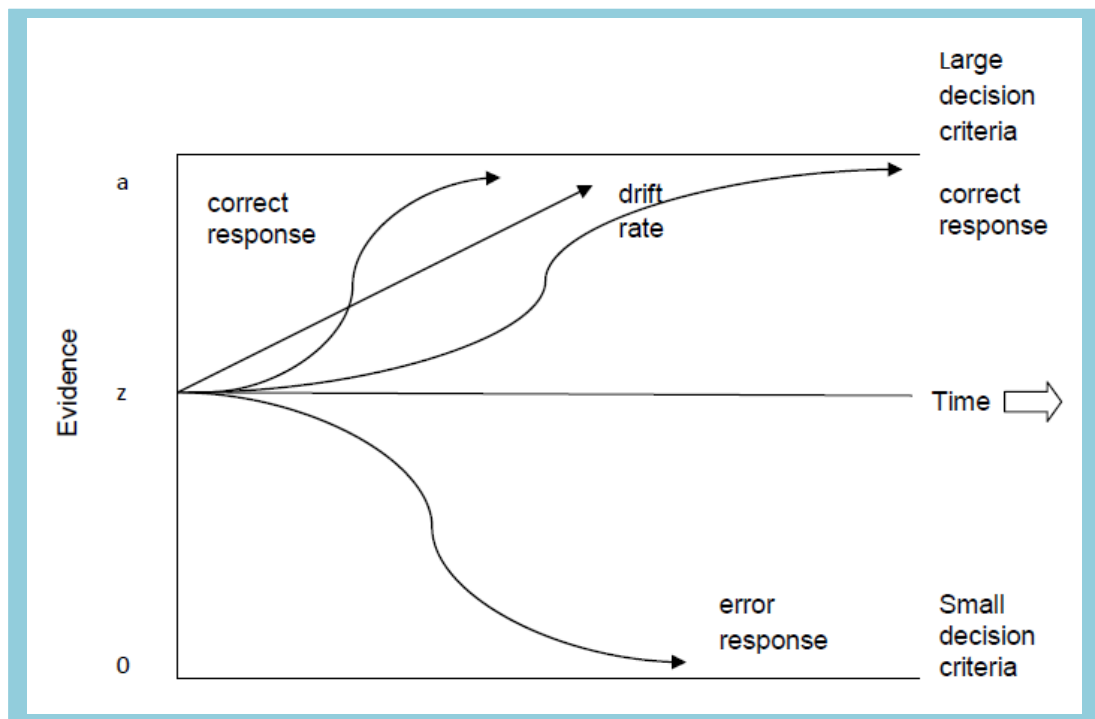
The diffusion model apportions parameter values to data from the relevant cognitive task and uses the parameter values representing the components of processing to interpret, for example, the effects of aging or sleep deprivation on performance [13-24]. The model (Figure 6.1) assumes that evidence from the stimulus is noisy and it is accumulated from a starting point (z) toward one or the other of the boundaries (a or 0). The mean rate of accumulation of evidence is called drift rate (v) and the assumption here is that the perceived numerosity is mapped into drift rate. Within-trial variability (noise) causes processes with the same drift rate to terminate at different times (producing RT distributions) and sometimes to terminate at the wrong boundary (producing errors). The values of the components of processing vary from trial to trial. Drift rate is assumed to be normally distributed across trials with SD η . Starting point is assumed to be uniformly distributed across trials with range s_z (which is equivalent to variability in decision criteria if the variability is not too large). Contaminant responses are modeled by assuming that on some proportion of trials (p_o), there is a random delay added to the decision RT, due to a moment's distraction, lack of attention, and other factors. The distribution is assumed to be uniform, but recovery of diffusion model parameters is robust to the actual form of the distribution [24] (Figure 6.1).

In signal detection theory, all variability would be attributed to the numerosity estimate (the estimate of whether the number of asterisks was larger or smaller than 50) with variability normally distributed across trials. In the diffusion model, this corresponds to variability in drift rate across trials. However, in the diffusion model, the different sources of variability, within-trial, starting point, and the nondecision component are separately identified when the model is fit to data. If predicted data are generated from

the model and the model is fit back to the predicted data, the parameter values are recovered accurately so that, for example, high variability in drift across trials is not misidentified as high variability in starting point [25]

For this numerosity discrimination experiment, we assume that drift rates are equal and opposite for small responses to small stimuli and large responses to large stimuli. For example, the drift rate for 31-35 asterisks has the same numerical value as the drift rate for 66-70 asterisks. However, subjects can have a bias in the zero point of drift, so we use a drift criterion to be added to each drift rate [25]. The addition of a positive drift criterion, for example, makes a drift rate for the condition with 31-35 asterisks larger numerically than the drift rate for the condition with 66-70 asterisks. For further details of the model, see Ratcliff and McKoon [24] and Ratcliff and Tuerlinckx [25].

Figure 6.1: An illustration of the diffusion model with starting point z , boundary separation a , and drift rate v .



6.2 Methods

The study protocol was approved by the Lothian medical research ethics committee, and all subjects gave informed consent for participation.

6.2.1 Subjects

Fourteen non-diabetic adult humans (5 male) were recruited from members of staff at the Royal Infirmary of Edinburgh. None had any relevant previous medical history of family history of diabetes, and none were taking regular medication (other than the oral contraceptive pill). All subjects had a corrected visual acuity of 6/6 or greater in both eyes, as measured with the Snellen chart. The median (inter-quartile range, IQR) age was 28 (27-35) years and the mean body mass index (SD) was 22.8 (2.61) kg m⁻². All of the subjects had above average intellectual ability as assessed by the National Adult Reading Test (NART) [26]. The mean (SD) NART correct score for 14 subjects was 41.5 (4.2).

6.2.2 Glucose clamp procedure

Each subject participated in two laboratory sessions, each separated by at least two weeks. The studies were conducted in the Clinical Research Facility of the Royal Infirmary of Edinburgh. During the experimental visits, subjects underwent a modified hyperinsulinaemic glucose clamp. The subjects completed both experimental conditions in a counterbalanced fashion. The subjects were not informed which condition was being studied at each visit.

Each session commenced at 8.00h following a 10 hour overnight fast. A Teflon cannula was inserted into the ante-cubital vein under local anaesthetic (2% lignocaine). This cannula was used to infuse human soluble insulin (Actrapid, Novo Nordisk pharmaceuticals, Crawley, UK) and 20% dextrose. A second cannula was inserted in a retrograde direction into a vein on the dorsum of the hand, which was placed in a heated blanket to arterialise the venous blood. Arterialised blood samples were obtained throughout the study for the measurement of whole blood glucose at the bedside using a glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, OH, USA). A modified hyperinsulinaemic glucose clamp technique (as described above) was used to maintain the blood glucose at predetermined levels. After a brief priming regimen, insulin was infused at a steady rate (based on whole body surface area) of $60 \text{ mU m}^{-2} \text{ min}^{-1}$ using an IVAC Signature Gold pump (Alaris Medical Systems, San Diego, CA); 20% dextrose was infused, also using a IVAC Signature Gold pump, at a variable rate depending on the blood glucose value. Arterialised blood glucose was measured initially at every 3 min, until a stable level had been achieved, and then at 5-minute intervals. At each laboratory session, arterialised blood glucose was initially stabilised at 4.5 mmol/L for a period of 30 minutes. Following this, the blood glucose concentration was either maintained at 4.5 mmol/L (euglycaemia) or lowered to 2.5 mmol/L (hypoglycaemia) and the neuropsychological tests administered. The subjects were not informed about their blood glucose concentration during any phase of the study. A period of 20 minutes was allowed to elapse between the baseline and the attainment of euglycaemia or hypoglycaemia to allow the blood glucose concentration to stabilise. The target glucose concentration was maintained for a further 10 minutes before the tests were administered and was maintain for a further 60 minutes while the tests were administered. At the end of the hypoglycaemia session the blood glucose was

restored to 4.5 mmol/L. Subjects were provided with a meal after completion of each condition.

6.2.3 Cognitive Function Tests

Signal Detection

For each trial, a number of asterisks between 30 and 70 were generated from a signal distribution. The asterisks were placed in random positions in a 10×10 array of blank characters on a computer screen (Sony Vaio TR2A Notebook). The subjects were asked to decide whether the number of displayed asterisks was "large" or "small" by pressing the "Z" key on the computer keyboard for the "large" group and the "?" key on the keyboard for the "small" group. Accuracy feedback was given on all trials. There were 12 blocks of 50 trials per session. For the data analyses, the numbers of asterisks were grouped into eight experimental conditions so that the mean RTs and accuracy values were about the same for the stimuli within a group.

Digit Symbol Substitution Test

The Digit Symbol Substitution Test consists of eight rows containing, in total, 200 small blank squares, each with a randomly assigned number from 1 to 9. Above these rows is a printed key that pairs each number with a different symbol. The subject is asked to fill in as many of the blank squares as possible with the appropriate symbol that matches the number above the box, in a time limit of 120 seconds. The score is the number of squares that are completed successfully within the time limit. This test is performed routinely during hypoglycaemia clamp experiments as a validity check on the cognitive effects of hypoglycaemia.

6.2.4 Symptom scores

The subjects scored the presence and intensity of symptoms of hypoglycaemia using the Edinburgh Hypoglycaemia Symptom Scale [27]. Symptoms of hypoglycaemia are classified as *autonomic* (hunger, palpitations, sweating, tremor), *neuroglycopenic* (confusion, drowsiness, difficulty concentrating, weakness) and *malaise* (nausea, headache). Subjects scored their symptoms of hypoglycaemia at baseline and at 45 minutes into the experimental period.

6.2.5 Statistical analysis

A general linear model (repeated measures analysis of variance [ANOVA]) was used, with order of session (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia) as a between-subjects factor, and condition (euglycaemia or hypoglycaemia) as a within-subject factor (repeated measure). A p value of less than or equal to 0.05 was considered to be significant. Effect sizes were calculated using eta squared (η^2). The principal measures of interest were the eta squared representing the proportion of the variance in the test scores accounted for by study condition (euglycaemia vs. hypoglycaemia). All analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

The diffusion model was fit to the data by minimizing a chi-square value with a general SIMPLEX minimization routine that adjusts the parameters of the model until it finds the parameter estimates that give the minimum chi-square value (26). The data entered into the minimization routine for each experimental condition were the 0.1, 0.3, 0.5, 0.7,

0.9 quantile RTs for correct and error responses and the corresponding accuracy values. The quantile response times and the diffusion model were used to generate the predicted cumulative probability of a response by that quantile response time. Subtracting the cumulative probabilities for each successive quantile from the next higher quantile gives the proportion of responses between adjacent quantiles. For the chi-square computation, these are the expected values, to be compared to the observed proportions of responses between the quantiles (i.e., the proportions between 0, 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0, which are 0.1, 0.2, 0.2, 0.2, 0.2, and 0.1) multiplied by the number of observations. Summing over $(\text{Observed}-\text{Expected})^2/\text{Expected}$ for all conditions gives a single chi-square value to be minimized. When there were too few observations (e.g., less than 5) for the extreme low error conditions for some of the subjects to form quantiles, a single chi-square value based on the response proportion alone was added to the overall chi-square value.

6.3 Results

6.3.1 Blood glucose

All results are reported as mean (SD) unless otherwise stated. A stable blood glucose plateau was achieved during both study conditions. The mean (SD) blood glucose concentration during the euglycaemia condition was 4.58 mmol/L (0.18), and during the hypoglycaemia condition it was 2.57 mmol/L (0.14).

6.3.2 Symptoms

Scores from the hypoglycaemia symptom questionnaire were significantly higher during hypoglycaemia for autonomic, $p = 0.004$, $\eta^2 = .515$, neuroglycopenic, $p = 0.001$, $\eta^2 = .584$, and malaise symptoms, $p < 0.001$, $\eta^2 = .706$, compared to scores obtained during euglycaemia.

6.3.3 Digit Symbol Substitution

The mean score of the Digit Symbol Substitution test deteriorated from 99.4 (19.4) during euglycaemia to 91.7 (21.7) during hypoglycaemia, $p = 0.009$, $\eta^2 = .451$. These findings establish that the hypoglycaemic intervention had the anticipated effects as demonstrated in similar hypoglycaemic clamp studies.

6.3.4 Reaction time and accuracy

The number of correct responses fell from 109.5 (2.8) during euglycaemia to 104.6 (4.8) during hypoglycaemia, $p = 0.002$, $\eta^2 = .558$. The mean reaction time also increased significantly during hypoglycaemia (691.2 (136.3) milliseconds) compared to 104.6 (4.8) milliseconds during euglycaemia, $p = 0.019$, $\eta^2 = .382$. The number of asterisks was grouped into eight categories (31-35, 36-40, 41-45, 51-55, 56-60, 61-65, 66-70) and the reaction times calculated and compared for each condition (euglycaemia versus hypoglycaemia) are displayed in Table 6.1.

Table 6.1: Reaction times for each condition and number of asterisks.

Asterisk	Euglycaemia	Hypoglycaemia	P value	η^2
31-35	623.8 (98.0)	696.8 (140.2)	0.03	.376
36-40	664.9 (113.5)	742.0 (170.4)	0.007	.499
41-45	728.5 (162.7)	791.0 (197.3)	0.09	.236
46-50	797.0 204.1	853.3 (245.5)	0.27	.102
51-55	760.2 (191.7)	776.4 (217.9)	0.70	.013
56-60	702.5 (191.7)	751.4 (183.5)	0.19	.141
61-65	634.5 (117.3)	682.1 (117.3)	0.12	.191
66-70	596.2 (132.6)	650.9 (132.6)	0.02	.359

6.4.5. Diffusion model

Responses from the first block of each session, short and long outlier RTs in all blocks, and the first response in each block were eliminated from data analyses. RT cutoffs used were a lower cutoff of 250 ms and an upper cutoff of 3000 ms. Summaries of the basic data are shown in Figure 6.2. The top panels show the proportion of 'large' responses as a function of the eight conditions (8 groups of numbers of asterisks) for the experimental (hypoglycaemic) and control (euglycaemic) conditions. The bottom panels show mean RT for 'large' and 'small' responses as a function of the number of asterisks for the two conditions. As the figures show, the probability of a 'large' response varies across the eight conditions from near 1 for stimuli with large numbers of asterisks to near 0 for stimuli with small numbers of asterisks. RT becomes longer for the conditions with intermediate numbers of asterisks.

Diffusion model fits. The diffusion model was fit to the data from each subject for each session. To display the fits, we computed the average over subjects for the quantile RTs and the response proportions for the data and, for the model, we generated predictions from the parameter values averaged over subjects.

We used quantile probability functions to display the quality of the fit of the model to the data. For each plot, the 0.1, 0.3, 0.5 (median), 0.7, and 0.9 quantiles of the RT distribution for each of the eight experimental conditions are plotted as a function of response proportion.

The only significant difference among model parameters as a function of the experimental manipulation is a reduction of mean drift rate from 0.290 to 0.211, $p < 0.05$ (Table 6.2). All other parameters were not significantly different.

Figure 6.2: Accuracy and mean RT as a function of condition

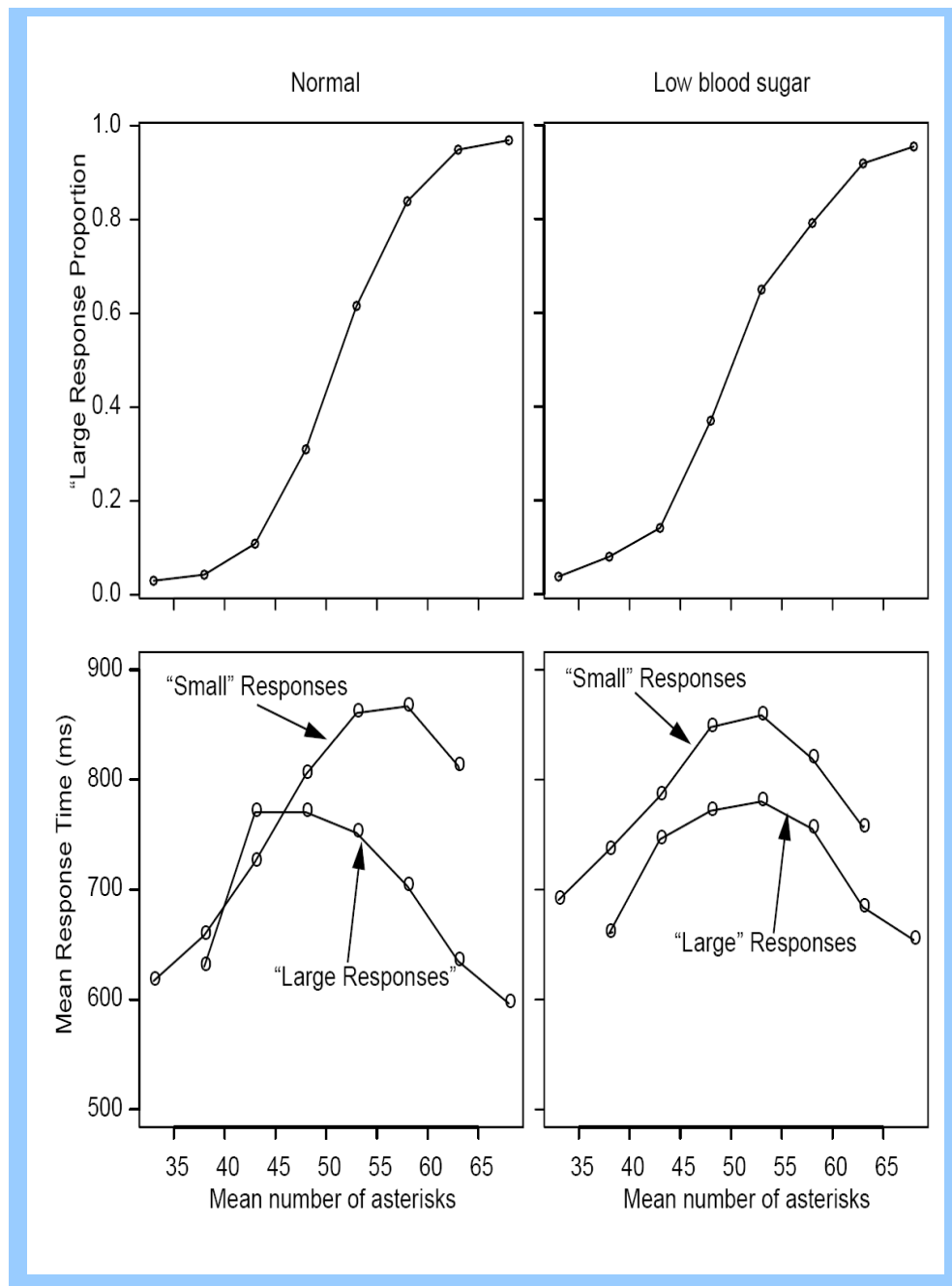


Table 6.2: The parameters of the model are: a =boundary separation, z is the starting point, T_{er} =duration of nondecision components of processing, h =standard deviation in drift across trials, s_z =range of the distribution of starting point (z) across trials, v_1 - v_4 are drift rates for the groupings shown in Figure 1, p_o =proportion of contaminants, v_c =drift criterion (a value added to drift rates for “small” responses and subtracted from drift rates for “large” responses), and s_t =range of the distribution of nondecision times across trials. x^2 is the goodness of fit index.

Condition	Statistic	a	T_{er} (sec)	η	s_z	v_1	v_2	v_3	v_4	p_o	s_t (sec)	v_c	Z	x^2
Eu	Fit to mean data	0.141	0.429	0.154	0.051	0.423	0.348	0.217	0.078	0.000	0.173	0.039	0.061	
	Average across subjects	0.146	0.431	0.156	0.065	0.460	0.379	0.234	0.085	0.001	0.170	0.039	0.064	80.
	SD across subjects	0.046	0.060	0.070	0.037	0.122	0.095	0.056	0.027	0.002	0.028	0.036	0.019	20.
Hypo	Fit to mean data	0.137	0.430	0.119	0.064	0.296	0.236	0.151	0.058	0.019	0.192	0.021	0.060	
	Average across subjects	0.148	0.413	0.137	0.073	0.334	0.270	0.174	0.068	0.004	0.176	0.025	0.065	117.
	SD across subjects	0.040	0.045	0.065	0.032	0.098	0.074	0.061	0.025	0.007	0.026	0.040	0.016	64.

6.4 Discussion

The results from this study show a powerful dissociation in terms of the diffusion model analysis. The model shows clearly that hypoglycaemia reduces drift rates by .08 out of .29 (with an even larger effect on the most accuracy conditions; the mean of \underline{v}_1 and \underline{v}_2 is reduced from 0.39 to 0.27). This indicates that hypoglycaemia affects central processing and not the quantity of evidence required to make a decision (boundary separation, \underline{a}), or peripheral and motor processes (nondecision component, \underline{T}_{cr}). This numerosity task was chosen because it is a reasonable control task in that there are not perceptual limitations (such as brief presentation time), no memory limitations as there might be in a memory task, and no limitations dependent on language knowledge. Drift rate in this task represents the estimate of numerosity of the stimulus derived from the array of asterisks. The variability in this estimate comes from the random arrangement of asterisks in the array. As far as we are aware, this is the first study to dissect the effects of hypoglycaemia in this way. The data now afford the opportunity to compare and contrast, precisely, the cerebral effects of hypoglycaemia and other factors.

Acute hypoglycaemia is a common side effect in people with insulin-treated diabetes, due to the non-physiological doses of insulin that are used in standard insulin regimens often leading to a mismatch between blood glucose and plasma insulin concentrations. The frequency of hypoglycaemia has been examined most extensively in people with type 1 diabetes, in whom mild (self-treated) hypoglycaemia occurs on average around twice weekly [28,29]. These episodes of acute hypoglycaemia lead to an impairment of mental performance and thus have important implications for work performance and the ability to carry out everyday tasks such as driving. However tasks such as driving require the integration of numerous cognitive functions including psychomotor,

information processing, attention and others. Hence, the precise neurobiological and cognitive bases of the decrement observed during testing remained poorly understood. As outlined in the introduction, prior research had more crudely indicated that visual and auditory processing showed decrements during hypoglycaemia and that peripheral nerve conduction was not affected (10-12). However, the dissection of specific decision making parameters that were studied here was not possible.

The dissociation of model parameters in the present study contrasts nicely with the effects of aging on processing in this task. Ratcliff, Thapar, and McKoon (30); Ratcliff, (31) observed that aging affected boundary separation and the nondecision component, but not drift rate. In contrast to aging, Ratcliff and Van Dongen (32) found that sleep deprivation affected drift rate to about the same degree as hypoglycemia, but also had a small effect on boundary separation. Further work is required to ascertain the potential additive effects of sleep deprivation and hypoglycaemia.

Strengths of the present study include the use of a powerful intervention in a within-subjects design. The task itself has a number of advantages for the beginning of an investigation. First, it has no perceptual or memory demands, the array is presented until the subject responds. In this sense it provides a useful baseline for other more cognitive or perceptual tasks. Second, by varying the number of asterisks, performance varies from near ceiling (100% accurate) to near floor (50% accurate). Third, the task has been successfully modelled in a number of domains such as in studies examining the effects of development, aging, sleep deprivation, and IQ on performance. It is a limitation of the present study however that only young, above average IQ participants were studied.

6.4.1 Conclusions

In conclusion, the present study is the first to locate the precise processing deficit that is associated with hypoglycaemia. This new information can lead in at least two directions, which are basic and applied. First, further work should bring together knowledge of the precise neurobiological effects of hypoglycaemia to link it with what we have now found to be the precise cognitive processing effects. This can help to reveal the neurobiology of thought processes at a mechanistic level that is rarely achieved. Second, understanding the cognitive processing parameters affected by hypoglycaemia and other central nervous system insults can lead to rational strategies for the amelioration of these effects.

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Chapter 7

An evaluation of methods of assessing impaired awareness of hypoglycaemia in Type 1 Diabetes

7.1 Introduction

Hypoglycaemia is a common side-effect of insulin treatment in type 1 diabetes and is detected by the subjective recognition of typical autonomic symptoms such as sweating, tremor and hunger, and neuroglycopenic symptoms such as drowsiness and difficulty in concentrating [1]. The early perception of these warning symptoms is fundamental to promote self-treatment and prevent progression to severe hypoglycaemia (SH) and the recognition of the onset of these premonitory symptoms constitutes “awareness” of hypoglycaemia [2-4]. However with increasing duration of insulin therapy, many people with type 1 diabetes experience a significant change in their awareness of hypoglycaemia associated with either a reduction in symptom intensity or a change in symptom profile [4-7]. Neuroglycopenic symptoms such as confusion, drowsiness and an inability to concentrate become predominant, while autonomic symptoms diminish in prevalence and intensity. This leads to the development of “impaired awareness of hypoglycaemia”, which is recognised to be an acquired syndrome associated with cerebral adaptation resulting from recurrent exposure to low blood glucose levels [5]. Impaired awareness of hypoglycaemia (IAH) is a prominent risk factor for SH and is associated with a three to six-fold increase in their frequency [8, 9].

This clinical syndrome varies in severity and is associated with significant morbidity, while also influencing medical fitness to drive. Accurate identification of affected individuals with impaired awareness of hypoglycaemia is therefore important to allow modification of their glycaemic targets and if necessary adjust insulin therapy to minimise the risk of severe hypoglycaemia. At present, three methods have been proposed for the assessment of awareness of hypoglycaemia [8,9,10]. A previous study

has examined the relationship between one of these methods using hypoglycaemia questionnaire, prospective blood glucose monitoring and glucose clamp studies to induce hypoglycaemia in assessing hypoglycaemia awareness [11]. However, the three methods currently available for clinical use have not been compared.

The present study was performed in a randomly selected cohort of people with type 1 diabetes to assess the concordance between these methods ascertaining the prevalence of IAH and whether they have equivalent sensitivity in identifying affected individuals.

7.2 Methods

The study protocol was approved by the Lothian medical research ethics committee, and all subjects gave informed consent for participation.

7.2.1 Subjects

A group of 140 adults with type 1 diabetes (defined by having two or more of the following characteristics: onset when aged less than 40 years, lean body habitus at time of diagnosis, commencement of insulin therapy at time of diagnosis or a history of diabetic ketoacidosis) who had attended the outpatient diabetes clinic over a period of 12 months, were selected at random. Eighty subjects completed the monitoring period (which will be discussed in full in section 7.2.2), 55 male; HbA1c mean (SD) 8.1% (1.4); age median (IQR) 47.5 (35.5-56.3); duration of diabetes, median (IQR), 20 years (9-31). Participants had to have had type 1 diabetes of more than two years duration and be over 16 years of age. Subjects were excluded if they were pregnant, had renal failure or were either unable or unwilling to do routine blood glucose monitoring. Information

about the presence of microvascular complications was obtained from their medical records.

7.2.2 Methods of assessing awareness of hypoglycaemia

The *Gold* method [8] poses the question: “do you know when your hypos are commencing?” The respondent then completes a 7-point Likert scale with 1 representing “always aware” and 7 representing “never aware”. A score of 4 or more implies impaired awareness of hypoglycaemia.

The *Clarke* method [9] comprises eight questions characterising the participant’s exposure to episodes of moderate and severe hypoglycaemia. It also examines the glycaemic threshold for, and symptomatic responses to, hypoglycaemia. A score of 4 or more implies impaired awareness of hypoglycaemia.

The *Pedersen-Bjergaard* method [10] requires the patient to respond to the question: “do you have symptoms when you have a hypo?” requiring the selection of one response from “always”, “sometimes” or “never”. Only patients who answer “always” are considered to have normal symptomatic awareness of hypoglycaemia, the others are designated as having impaired or absent awareness.

Awareness of hypoglycaemia was assessed using each of the three methods (*Gold*, *Clark*⁹ & *Pedersen-Bjergaard*). All participants completed a questionnaire for each method in the presence of a member of the research team who could, if necessary, explain the terminology used and ensure the complete comprehension of the questions by the participants. Clinical information was obtained on the insulin regimen being used and on concurrent medications. The participants were then asked to perform capillary blood glucose measurements four times daily, prospectively over a four-week period,

testing before meals and at bedtime and the results were recorded in a diary. When any blood glucose value was recorded as being less than 3 mmol/L, the subjects were asked to complete a validated symptom questionnaire (the Edinburgh Hypoglycaemia Score) to document the nature and the intensity of the hypoglycaemic symptoms that were experienced at that time [1]. Information was also documented as to whether the onset of the episode of hypoglycaemia was recognised by the participant or by an independent observer and the treatment used. The completed diaries and information sheets were returned to the investigators at the conclusion of the four-week monitoring period.

7.2.3 Statistical analyses

All analyses were performed using SPSS version 12.0 for Windows. The Kolmogorow-Smirnov test was applied to check the normality of the variables, depending on which, differences between groups were analysed using either the two-sample t test or Mann-Whitney-U test. To assess the linear relationship between two variables a Spearman correlation coefficient was calculated. Differences between the groups were analysed using χ^2 . A p value ≤ 0.05 was considered to be significant. All results are reported as mean (SD) unless otherwise stated.

7.3 Results

Of the 140 participants recruited, 80 completed the blood glucose-monitoring period for one month and returned the results. Those who completed the study ($n = 80$) were significantly older than those who did not ($n = 60$), (47.6 (12.7) vs. 41.1 (12.6) years, $p = 0.04$). No differences in duration of diabetes ($p = 0.7$) or in glycaemic control ($p = 0.35$) were observed between these two groups.

7.3.1 Prevalence of impaired awareness of hypoglycaemia

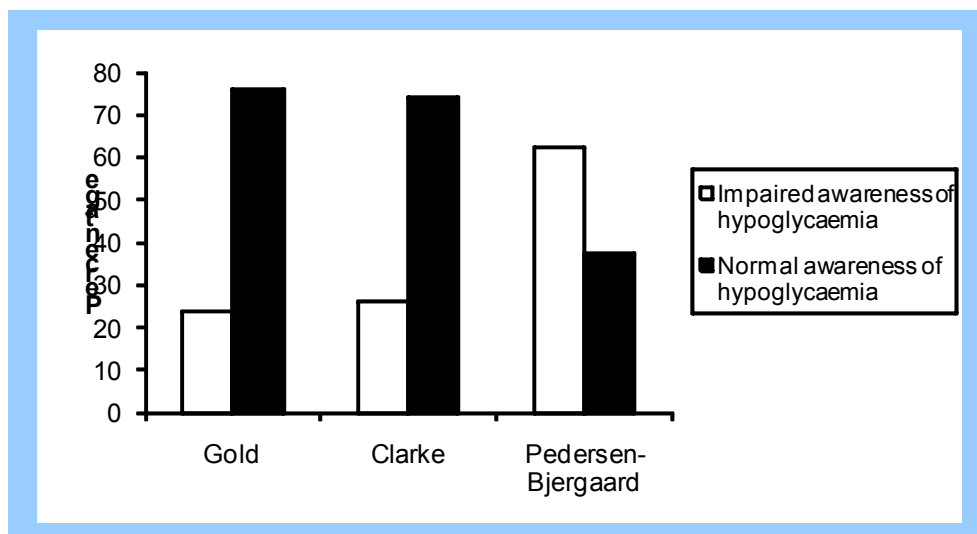
The prevalence of impaired awareness of hypoglycaemia as identified by the *Gold*, *Clarke* and *Pedersen-Bjergaard* methods were 24%, 26% and 62.5% respectively (Table 7. 1), (Figure 7.1). A strong association, using Spearman's test was found between the Gold and Clarke methods for identifying impaired awareness ($r_s = 0.868$, $p = 0.001$).

Those with IAH identified by the *Gold* method ($p = 0.01$) and the *Clarke* method ($p = 0.004$) were significantly older than those patients who were designated as having had normal awareness when using either of these two methods (Table 1). No age difference was observed between the two groups of aware and IAH patients applying the *Pedersen-Bjergaard* method ($p = 0.10$). The duration of diabetes was significantly longer in the IAH group of patients with all three methods but no statistical difference was observed in HbA1c between the two groups, subdivided by state of awareness.

Table 7.1: Clinical characteristics of participants with type 1 diabetes by methods of assessment of awareness of hypoglycaemia

Method of evaluation	Gold method⁸			Clarke method⁹			Pedersen-Bjergaard method¹⁰		
Awareness	NAH	IAH	p	NAH	IAH	p	NAH	IAH	p
Number (%)	61 (76%)	19 (24%)	-	59 (74%)	21 (26%)	-	30 (37.5%)	50 (62%)	-
Age (years) (Median, IQR)	44 (33-53)	56 (47-63.5)	0.001	45 (33-53)	56 (44-63)	0.007	42 (32-54)	49 (38-57.5)	0.09
Gender (M/F%)	52.6 / 47.3	42.9/ 57.1	0.93	52.6 / 47.4	42.9/ 57.1	0.49	51.7 / 48.3	49/ 51	0.44
Duration (years) (Median, IQR)	18.5 (7.5-27.5)	27 (13-36.5)	0.04	16 (7-25.3)	27 (20-38)	0.003	13 (7-21)	24 (10-34.3)	0.03
Daily insulin dose (IU)	55.8 (32.7)	50.0 (21.8)	0.22	56.2 (33.2)	50.0 (22.8)	0.16	50.4 (21.5)	54.0 (33.6)	0.64
> 4 injections/ day	68.4 %	85.7 %	0.51	77.6%	95%	0.09	75 %	86.7 %	0.85
HbA1c (%)	8.3 (1.4)	7.8 (1.3)	0.23	8.2 (1.4)	8.1 (1.2)	0.75	8.2 (1.4)	8.1 (1.3)	0.55

Figure 7.1: Frequency of awareness of hypoglycaemia by methods of assessment of awareness of hypoglycaemia.



7.3.2 Frequency of biochemical hypoglycaemia

The frequency of recorded episodes of biochemical hypoglycaemia is shown in Table 7.2. The patients designated as having IAH using the *Gold* and *Clarke* methods reported a significantly higher number of episodes of biochemical hypoglycaemia over the four-week monitoring period than those patients considered to have normal awareness of hypoglycaemia. No statistical difference in mean number of hypoglycaemia episodes was observed between the two sub-groups using the *Pedersen-Bjergaard* method ($p = 0.06$). The mean number of home blood glucose measurements between 2.5-2.99 mmol/L and <2.5 mmol/L differed significantly between the *Gold* and *Clarke* subgroups (IAH and normal awareness) but differences in the frequency of hypoglycaemia were not apparent using the *Pedersen-Bjergaard* method ($p = 0.11$). During the four-week period of prospective blood glucose monitoring, the reported intensity of autonomic symptoms was lower during episodes of biochemical hypoglycaemia in those who had IAH as identified using the *Clarke* and *Gold* methods, compared to patients designated as having normal awareness. No symptomatic differences were observed between the groups identified using the *Pedersen-Bjergaard* method ($p = 0.22$). No statistical differences between groups with IAH and normal awareness were observed in the recorded intensity of neuroglycopenic symptoms during episodes of hypoglycaemia with any of the methods evaluated.

In the present study, the mean incidence of severe hypoglycaemia reported to have occurred during the preceding year was statistically different between those identified as having IAH compared to those with normal awareness using all of the three methods.

Table 7.2: The frequency of episodes of biochemical hypoglycaemia over the 4 week period and recollected total number of episodes of severe hypoglycaemia (SH) during the previous year.

Method of assessment	Gold [8]			Clarke [9]			Pedersen-Bjergaard [10]		
Awareness	NAH	IAH	p	NAH	IAH	p	NAH	IAH	p
From record sheets (%)									
Total biochemical glucose values < 3.0mmol/L	3.49 (3.64)	7.62 (5.35)	0.003	3.40 (2.65)	7.86 (5.10)	0.001	3.31 (3.51)	5.37 (4.90)	0.06
Biochemical glucose values 2.5-2.9 mmol/l	2.38 (2.64)	4.14 (2.92)	0.02	2.33 (2.65)	4.29 (2.81)	0.006	2.26 (2.55)	3.24 (2.91)	0.11
Biochemical glucose values <2.5mmol/l	1.11 (1.71)	3.47 (3.81)	0.01	1.02 (1.67)	3.57 (3.80)	0.005	1.05 (1.51)	2.08 (3.11)	0.11
Severe hypoglycaemic reactions	0 (0)	0.1 (0.7)	0.10	0.05 (0.47)	0 (0)	0.55	0 (0)	0.05 (0.43)	0.44
Autonomic symptoms	2.88 (1.06)	2.09 (0.99)	0.005	2.96 (1.05)	1.89 (0.79)	0.001	2.87 (1.08)	2.54 (1.09)	0.22
Neuro-glycopenic symptoms	2.25 (1.02)	2.45 (1.14)	0.47	2.29 (1.06)	2.35 (1.06)	0.83	2.12 (1.00)	2.41 (1.08)	0.27
% of episodes recognised by participant	85%	50%	0.014	90%	47.6%	0.013	92%	62%	0.013
% of episodes identified from meter	13%	48%	0.26	9%	50.0%	0.261	8%	36%	0.395
% of episodes recognised by someone else	2%	2%	0.39	1%	2.4%	0.31	0%	2%	0.31
From questionnaire									
Incidence of SH (episodes per patient year)	0.07 (0.32)	1.57 (2.82)	0.001	0.05 (0.29)	1.62 (2.80)	0.001	0 (0)	0.76 (1.98)	0.04
Prevalence of SH	5%	53%	-	5%	57%	-	0%	26%	-

7.4 Discussion

Impaired awareness of hypoglycaemia is a frequent problem in people with type 1 diabetes, becoming more common with increasing duration of diabetes [5,7,13] and being associated with strict glycaemic control [4,7]. It is not an “all or nothing” phenomenon and usually develops gradually over months to years. No clear consensus exists for the definition of impaired awareness of hypoglycaemia, but clinical assessment should be based on the everyday experiences of people treated with insulin, and not on observations carried out in the artificial setting of experimentally-induced hypoglycaemia in a laboratory, which does not simulate normal experience. Symptom generation differs during the induction of hypoglycaemia using a clamp technique than hypoglycaemia that occurs during waking hours in a community setting. Furthermore, symptoms can be modified and diminished in intensity in certain circumstances but these isolated events do not constitute impaired awareness of hypoglycaemia, so the diagnosis of the established syndrome should not be made on this basis.

Three methods are currently available to assess symptomatic awareness of hypoglycaemia. In the present study these methodologies have been evaluated for their sensitivity in identifying impaired awareness of hypoglycaemia and their mutual concordance. In a present cohort of randomly selected adults with type 1 diabetes, equivalent prevalence of impaired awareness (24% and 26%) were obtained with two of the methods (*Gold* and *Clarke*) but differed considerably from the prevalence observed using the method of *Pedersen-Bjergaard* (62.5%). Previous population surveys have indicated that impaired awareness of hypoglycaemia affects approximately one-quarter of unselected adult populations with type 1 diabetes which is consistent with the estimates using the Gold and Clarke methods in the present survey [5, 13, 14].

In the present study, differences were also apparent between the methods with respect to patients who were identified as being at high risk of impaired awareness. Applying the *Clarke* and *Gold* methods, the patients that the methods identified as having IAH were older, had a longer duration of diabetes and had a history of experiencing more episodes of severe hypoglycaemia during the year preceding the study, which is consistent with the recognised features of this syndrome [8, 9, 13]. The frequency of mild (and asymptomatic) hypoglycaemia episodes was also significantly higher in the groups with impaired awareness identified by the methods of Gold and Clarke and these patients had significantly lower autonomic and higher neuroglycopenic symptom scores during hypoglycaemia compared to those with intact awareness, which are recognised features of this syndrome. The difference in neuroglycopenia scores between these two groups failed to reach statistical significance, but this symptom profile is typical of patients with impaired awareness. The absence of a statistical difference in relation to the neuroglycopenia symptom scores between the two sub-groups reflects the arbitrary cut-off value of blood glucose of less than 3.0 mmol/L that was chosen for the subjects to record their responses to hypoglycaemia. This coincides approximately with the glycaemic threshold (3.2 – 2.8 mmol/L) for the generation of the symptomatic response to hypoglycaemia whereby deprivation of glucose causes a rapid deterioration in cognitive function with the subsequent generation of neuroglycopenic symptoms [15]. The *Pedersen-Bjergaard* method not only recorded a much higher prevalence of IAH but was less discriminating and identified only those patients who had a long duration of diabetes and a history of previous SH as characteristics relevant to those who had impaired symptomatic awareness.

The *Gold* and *Clarke* methods both validated the accuracy of their evaluation questionnaires by using a prospective period of home blood glucose monitoring (varying from 6-12 months), during which the frequency and the subjective evaluation of individual patients to

perceive the onset of symptoms to hypoglycaemia were recorded and compared with the assessment based on the questionnaire. By contrast, the much simpler *Pedersen-Bjergaard* method was evaluated by administering their simple questionnaire to a group of individuals with type 1 diabetes who were then followed prospectively to record their prevalence of SH. Those designated as having “impaired awareness” and “unawareness” did have an overall higher frequency of severe hypoglycaemia compared to the group with “normal awareness”, and this was advocated as justification of this method of assessment of hypoglycaemia awareness status.

The present study also demonstrated a strong correlation between the *Clarke* and *Gold* methods ($r_s = 0.868$, $p = 0.001$). The *Clarke* method has also been externally validated by a different group of investigators, who found that *Clarke's* self-report questionnaire achieved reasonable agreement with the autonomic symptom threshold during experimental hypoglycaemia (sensitivity 66.7%, specificity 85.7%), although prospectively recorded home blood glucose readings were not related to the hypoglycaemic clamp findings [11]. However the authors conceded that these disparate findings result from using an arbitrary cut-off blood glucose of $<3.9\text{mmol/L}$ for the recognition of hypoglycaemic symptoms and suggested that this could be rectified by applying a value of $<3.0\text{mmol/L}$, as was applied in the present study.

When methods that utilise questionnaires are used to ascertain awareness of hypoglycaemia, some overlap will occur and no currently available method can be considered to totally reliable. However, the *Pedersen-Bjergaard* method to identify patients with impaired awareness of hypoglycaemia represents too simplified an approach to a complex clinical condition and appears to be insensitive and insufficiently discriminating, so over-estimating the prevalence of this clinical syndrome. It cannot therefore be endorsed for routine clinical use.

In conclusion this present evaluation of methods of assessing symptomatic awareness demonstrates that, for both clinical and research use, the *Clarke* and *Gold* methods should be used preferentially, either separately or in combination, to identify people with type 1 diabetes who have impaired awareness of hypoglycaemia.

7.5 References

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Chapter 8

Frequency of hypoglycaemia in adults with and without impaired awareness of hypoglycaemia

8.1 Introduction

Hypoglycaemia is a common and disruptive side effect of insulin treatment for type 1 diabetes. Hypoglycaemia may be “mild” where the patient can identify falling glucose levels and take appropriate corrective action (ingestion of carbohydrate) or severe when glucose levels fall to a level where cognitive impairment prevents them from being able to treat themselves and they require third party assistance. Subjective recognition of the symptoms of hypoglycaemia is fundamental to enable effective self-management of hypoglycaemia and prevent progression to severe hypoglycaemia (SH)[1,2]. The ability to perceive the onset of these symptoms is fundamental to normal “awareness” of hypoglycaemia. Previous studies have suggested that impairment of awareness is associated with a three to six-fold increase in the frequency of episodes of severe hypoglycaemia [2-4].

Antecedent hypoglycaemia has been implicated in the pathogenesis of impaired awareness of hypoglycaemia (IAH) by blunting symptomatic and hormonal responses to subsequent hypoglycaemia [5,6]. However, once this syndrome has developed it is unclear whether it is associated with a substantial increase in the frequency of mild biochemical hypoglycaemia. A 12 month prospective study, which compared 31 patients with type 1 diabetes who had normal awareness with 29 who had IAH, observed no difference in the total number of episodes of mild hypoglycaemia between the two groups [4].

Continuous glucose monitoring (CGM) provides near continuous information about interstitial tissue glucose levels, allowing an examination of exposure to low blood glucose concentrations over several days. This raises two questions. Firstly, are subjects with type 1 diabetes that have impaired awareness of hypoglycaemia exposed to more asymptomatic biochemical hypoglycaemia? Secondly, if so, is a standard monitoring period of 4-5 days using CGM sufficient to identify those with a higher rate of biochemical hypoglycaemia and impaired awareness of hypoglycaemia?

8.2 Methods

Each participant was provided with similar capillary glucose testing devices (Medisense G glucose meter, Abbott Laboratories, Abbott Park, IL, USA) and were requested to perform one four-point blood glucose profile daily, (three measurements before meals and one at bedtime) in a 24 hour period, once a week for the duration of the study (BGM data). They were also asked to record all episodes of self-reported hypoglycaemia (a capillary blood glucose reading ≤ 3.5 mmol/l, with or without symptoms) and severe hypoglycaemia (SH), (any event requiring assistance for recovery), during this time. Subjects were contacted monthly by members of the research team to collect both the BGM and self-reported hypoglycaemia data. After screening, participants underwent CGM, (Medtronic CGMS, Medtronic, Minneapolis, MN, USA) for at least 96 hours (mean 5 days). Monitoring devices were calibrated five times per day.

8.2.1 Subjects

The data analysed had been collected as part of the UK Hypoglycaemia Group Study [7]. A group of 95 adults with type 1 diabetes as defined by WHO criteria [8], were studied prospectively over a one-year period, and their characteristics are shown in table 8.1. Awareness of hypoglycaemia was characterised at screening using the method of Gold et al [4], a validated scoring system (as discussed in Chapter 7) in which subjects are asked to respond to the question: “do you know when your hypos are commencing?” on a 7-point Likert scale, with 1 representing “always aware” and 7 representing “never aware”. A score of 4 or more has been shown to be consistent with diminished symptomatic responses to hypoglycaemia (impaired awareness of hypoglycaemia) [4,9], and this method has been shown in Chapter 7 to have close concordance[9] with the method described by Clarke et al [7]. Exclusion criteria were HbA1c $>9\%$, pregnancy, advanced complications of diabetes,

severe systemic disease or malignancy, a history of seizures unrelated to hypoglycaemia, or inability to give informed consent. The study had medical ethics committee approval, and written informed consent was obtained from all participants.

8.2.2 Analysis of CGM

To avoid artefacts such as sensor failure being falsely identified as hypoglycaemia, two observers independently analysed each CGM trace (software version 1.7a), and a third observer reconciled any discrepancies. Data that did not meet stringent validity criteria were excluded (mean absolute error (MAE) $< 28\%$, at least 3 calibrations / 24 hrs and data located between two paired calibration points). Total duration of valid data was calculated and periods of low interstitial glucose (LIG) were stratified into episodes ≤ 3 mmol/l (LIG_{3.0}) and those ≤ 2.2 mmol/l (LIG_{2.2}). LIG was defined as an episode of sensor glucose below the threshold value (2.2 or 3.0 mmol/l respectively) for at least 20 min and the episode was completed once the glucose remained above the respective threshold value for a further 20 min [8]. We selected these glucose levels on the basis of previous observations that cognitive function deteriorates at around an arterialised blood glucose level of 3.0 mmol/l [10,11] and because 2.2 mmol/l, which is the limit of detection of a low interstitial glucose using a CGM device, has been shown to correlate with clinical hypoglycaemia[12].

8.2.3 Biochemical analysis

HbA_{1c} was DCCT aligned and measured by ion exchange high performance liquid chromatography (Tosch Automated Glycohaemoglobin Analyser). C-peptide was measured by DAKO-ELISA kits (Dakocytomation Ltd, Ely, Cambridgeshire) and serum

Angiotensin-Converting Enzyme (ACE) was measured by a rapid spectrophotometric method (Sigma Diagnostics, St Louis, MO, USA).

8.2.4 Statistical analysis

Initial comparisons between groups (NAH versus IAH) for continuous variables were performed using the two-sample t test or Mann-Whitney-U test where necessary. For categorical variables differences in proportions between the groups were compared using the Chi-squared test with continuity correction or Fisher's exact test when necessary. Many individuals experienced no episodes of hypoglycaemia (either self-reported or biochemical) during monitoring and relatively few experienced multiple episodes. Thus in order to compare rates of hypoglycaemia between groups a negative binomial model was fitted to the data as this took into account both the exposure time and the excess of individuals who had no events during monitoring periods [13]. All analyses were performed using SPSS version 14.0 for Windows and STATA version 8. A p-value ≤ 0.05 was considered to be significant and no adjustments were made for multiple comparisons [14].

8.3 Results

Data were analysed from 95 subjects who completed the study and baseline characteristics are shown in Table 8.1. Those identified as having IAH ($n = 21$) had a significantly longer duration of diabetes compared to those with normal awareness of hypoglycaemia, (median (IQR) 34(16-44) vs. 4 (2-27) yrs; $p < 0.001$) but no significant differences were observed between the groups with regard to age ($p = 0.12$), serum ACE levels ($p = 0.28$), gender ($p = 0.07$) or glycaemic control as measured by HbA1c ($p = 0.08$) (Table 6.1). Median (IQR) duration of follow up was 48 (43-52) weeks.

8.3.1 Frequency of Severe Hypoglycaemia

Over the course of the study, patients with IAH had a 3-fold higher incidence of SH (requiring third party assistance) compared to those with NAH (incidence rate ratio (IRR): 3.37, 95% CI: 1.3 to 8.7; $p = 0.01$), (Table 8.2, Figure 8.1).

8.3.2 Frequency of hypoglycaemia on weekly capillary blood glucose monitoring

Subjects with IAH recorded a 1.6 fold higher rate of biochemical hypoglycaemia (capillary glucose of ≤ 3.5 mmol/l on weekly HBGM) than those patients with NAH (IRR: 1.63 (95% CI: 1.09 to 2.44); $p = 0.02$), (Figure 8.2).

Table 8.1: Clinical characteristics of participants with type 1 diabetes by hypoglycaemia awareness status. Values are mean (SD).

	Normal Awareness (74)	Impaired Awareness (21)	P- value
Age(years)	47.1 (13.4)	52.2 (12.1)	0.12
BMI (kg/m ²)	26.8 (4.9)	27.9 (4.4)	0.33
Gender (%male)	68.9	47.6	0.07
Duration of diabetes (years)	14.4 (15.1)	30.6 (16.9)	< 0.001
Daily insulin dose (units)	50.4 (25.8)	47.1 (22.7)	0.60
HbA1c (%)	7.4 (1.0)	7.9 (0.6)	0.08
Fasting C-peptide (pmol/l)	0.32 (0.3)	0.25 (0.3)	0.42
Serum ACE	42.9 (55.3)	29.6 (20.5)	0.28

Table 8.2: Comparison of hypoglycaemia between individuals with type 1 diabetes who have normal hypoglycaemia awareness and those with impaired awareness.

	Normal awareness	Impaired awareness	P value
Severe hypoglycaemia (per month)	0.13 (0.34)	0.41 (0.7)	0.03
Biochemical hypoglycaemia (per week)	0.82 (0.86)	1.29 (1.01)	0.02
Daytime CGM Lig 3.0 (mean)	1.43 (0-4.22)	3.47 (0-6.9)	0.12
Daytime CGM Lig 3.0 (duration/mins)	58 (40-90)	74 (37-107)	0.54
Nighttime CGM Lig 3.0 (mean)	1.18 (0-3.5)	2.33 (0-3.57)	0.26
Nighttime CGM Lig 3.0 (duration/mins)	110 (80-242)	79 (58-219)	0.17
Daytime CGM Lig 2.2 (mean)	0 (0-1.52)	1.16 (0-3.1)	0.26
Daytime CGM Lig 2.2 (duration/mins)	48 (30-100)	80 (38-135)	0.74
Nighttime CGM Lig 2.2 (mean)	0 (0-1.75)	1.17 (0-148)	0.96
Nighttime CGM Lig 2.2 (duration/mins)	126 (50-174)	103 (45-208)	0.93

Figure 8.1: Comparison of monthly rates of severe hypoglycaemia (self-reported) between subjects with Normal Awareness of Hypoglycaemia (NAH) and Impaired Awareness of Hypoglycaemia (IAH)

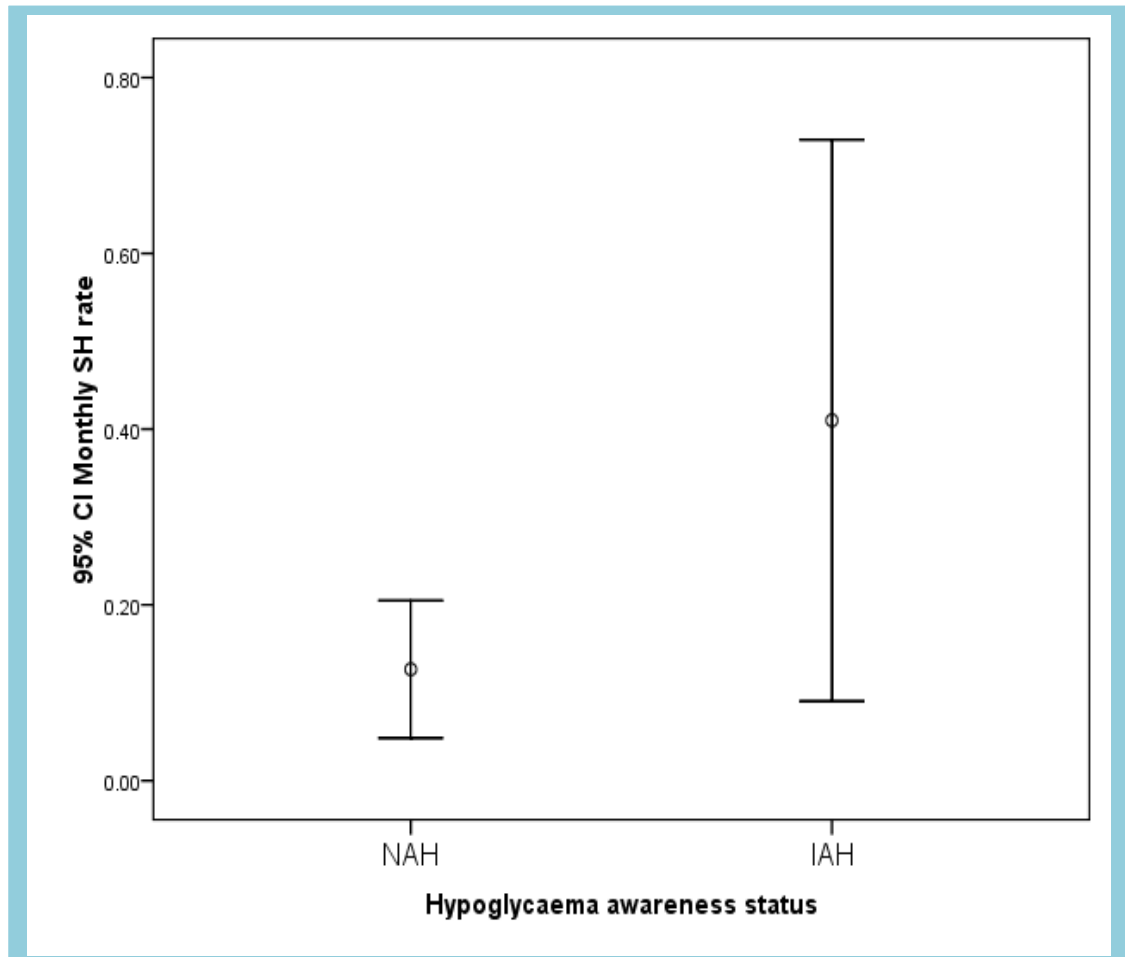
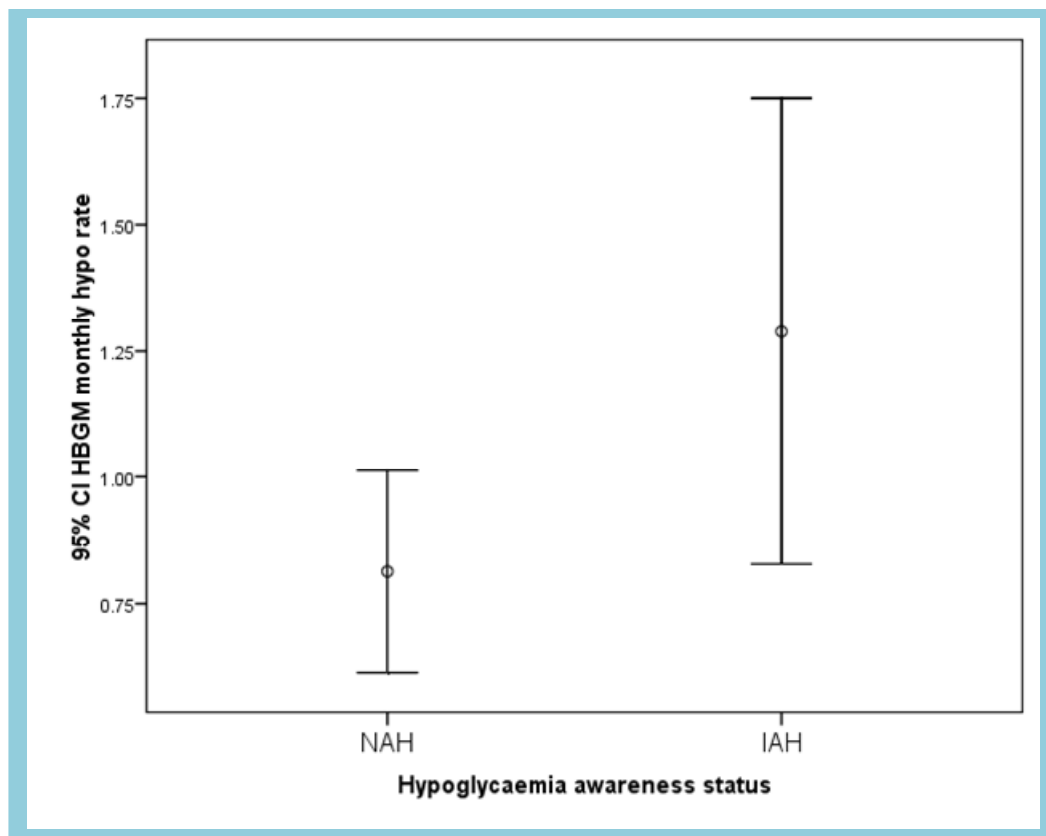


Figure 8.2: Comparison of monthly rates of biochemical hypoglycaemia between subjects with Normal Awareness of Hypoglycaemia (NAH) and Impaired Awareness of Hypoglycaemia (IAH).



8.3.3 Frequency of patient reported hypoglycaemia

No differences were observed between NAH and IAH subjects in the rate of patient reported symptomatic hypoglycaemia (NAH vs. IAH 2.99(4.07) vs. 2.64(2.83) episodes / week; $p=0.7$). Rates of hypoglycaemia across groups were consistent across the duration of the study (between 2.4 and 3.5 episodes / pt / month).

8.3.4 CGM data

All patients completed at least 96 hours of CGM data. Mean (SD) valid time was 4.9 (0.1) days/subject.

8.3.4 Frequency and duration of hypoglycaemia on CGM

No significant differences in frequency of biochemical hypoglycaemia measured as either $LIG_{3.0}$ or $LIG_{2.2}$ were observed [IRR for $LIG_{3.0}$: 1.37 (95% CI: 0.94 to 2.00); $p = 0.10$; IRR for $LIG_{2.2}$: 1.26 (95% CI: 0.71 to 2.23); $p = 0.44$]. No difference was observed in the median duration of each episode of hypoglycaemia between NAH and IAH (86 (53 to 145) vs. 79 (38 to 121) mins / episode; $p=0.37$ for $LIG_{3.0}$) or the percentage of total valid time with the sensor glucose ≤ 3.0 mmol (3.9 vs. 4.8%; $p=0.59$).

When divided into day-time (08:00 – 24:00) and night-time (00:00 – 08:00) no difference was found between NAH or IAH either in the number of episodes of $LIG_{3.0}$ (day-time: 1.43 (0-4.22) vs. 3.47(0-6.98) episodes /week; $p=0.12$; night-time: 1.18(0-3.5 vs. 2.33(0-3.6) episodes/week; $p=0.26$) or in the duration of time spent below a sensor glucose of 3.0 mmol/l (day-time: 58 (40-90) vs. 74(37-110) mins / episode) $p=0.54$; night-time: 110(80-242) vs. 79(58 to 219) mins/ episode; $p=0.17$) (Table 8.2).

8.3.5 Sensor glucose during symptomatic hypoglycaemia

90 episodes of patient reported hypoglycaemia (74 in IAH patients) were reported for which there was simultaneous valid CGM data. Mean capillary glucose during patient reported hypoglycaemia was lower in IAH than NAH (2.8(0.5) vs. 2.4(0.5) mmol/l; $p=0.005$) although there was no difference in nadir sensor glucose (lowest sensor glucose within 2 hours of the reported time of hypoglycaemia). Approximately 10% of episodes of LIG were associated with symptomatic hypoglycaemia. However, between 80 and 90% of symptomatic hypoglycaemia was associated with low sensor glucose.

8.4 Discussion

In this prospective observational study of unselected patients from 6 different centres in the UK, patients with type 1 diabetes with impaired awareness of hypoglycaemia (IAH) experienced a three-fold higher rate of severe hypoglycaemia, and a 1.6 fold higher rate of biochemical hypoglycaemia as measured by weekly 4-point home glucose monitoring compared to patients with normal hypoglycaemia awareness. However, no significant differences in biochemical hypoglycaemia (low interstitial glucose), measured using CGM, were detected between the two groups.

Patients with IAH had a longer duration of diabetes and a higher risk of developing severe hypoglycaemia than those with normal awareness, consistent with previous reports [3,4,15,16]. The prevalence of IAH in the present study (22.1%) was also comparable to another recent estimate [17] confirming that the present study population was typical of an unselected cohort of adults with type 1 diabetes. The majority of the participants in the present study were treated with intensive insulin regimens and had received structured

education on diet and insulin dose adjustment such as the Dose Adjustment For Normal Eating (DAFNE) programme, which can reduce the frequency of severe hypoglycaemia[18].

In the present study, we quantified the frequency of biochemical hypoglycaemia using two separate techniques. Although a higher frequency of biochemical hypoglycaemia was observed in those with IAH using weekly four point capillary glucose monitoring (BGM), no differences either in frequency or duration of LIG were observed with five days of CGM. The disparate results obtained between these two methods were unexpected and contrast to those reported by Kubiack et al [19] in which a two-fold higher frequency of biochemical hypoglycaemia (assessed using CGM) was reported in patients with IAH. However, various methodological differences between the two studies may explain these seemingly contrary findings. The present study examined a larger number of patients, in whom hypoglycaemia awareness was determined using an established scoring system, and in contrast to the study by Kubiak et al[19], mean HbA1c was similar between the patients with and without IAH. In addition, our study is more representative of everyday life as patients were studied in their home environment rather than in a hospital setting[19]. Kubiak et al used a higher glucose cut off of $\leq 3.3\text{mmol/l}$ to define hypoglycaemia and did not exclude CGM data that did not satisfy validity criteria, so may have overestimated the incidence of hypoglycaemia. Data from the UK Hypoglycaemia Group Study[8,20] and from the DirecNet group [21] suggest that a sensor glucose level of 3.0mmol/l provides the optimum sensitivity and specificity for true hypoglycaemia. At higher glucose cut-off values, specificity is lost whereas at lower glucose levels sensitivity is affected.

The 1.6 fold higher frequency of biochemical hypoglycaemia (with HBGM) in patients with IAH is similar to that seen in a study by Geddes et al[9], (Study 7), who reported a 2.2 fold higher incidence of biochemical hypoglycaemia with HBGM in patients with type 1

diabetes and IAH compared to those with NAH. HBGM has its limitations, in that the frequency of hypoglycaemia is dependent on the frequency and timing of testing. Many patients with IAH test their blood glucose more frequently than other patients, and times of testing may be biased towards times when hypoglycaemia is more likely. These problems were overcome in the present study by standardising the number of tests to a weekly 4-point glucose profile at fixed times before meals and at bedtime. However, the trade off is that any inter-prandial, and in particular nocturnal, hypoglycaemic event may be missed and may therefore cause underestimation of the true difference between the groups. Participants were contacted by telephone on a monthly basis to ensure that data collection of the weekly capillary blood glucose data was complete and that all episodes of severe hypoglycaemia had been identified and recorded.

While the data from recall of severe hypoglycaemia and analysis of BGM records are consistent with what was anticipated, the inability to detect any difference in hypoglycaemia with CGM between those with normal and impaired hypoglycaemia awareness was unexpected. Several possible explanations can be considered. The most likely is insufficient duration of CGM to detect a difference in hypoglycaemia exposure between these groups, effectively introducing a type 2 error. Despite being encouraged to continue with their normal routine, participants might deliberately have avoided potential precipitants of hypoglycaemia such as physical exercise or ingestion of alcohol during the five days of CGM, measures that they would have been less likely to sustain over the longer period of the HBGM analysis. It is also possible that hypoglycaemia may be more common at the times designated for testing capillary glucose in those with IAH compared to people with normal awareness. Given the sample size, it is unlikely that we have missed a large difference in LIG rates between the two groups. This information is important, as in clinical practice CGM is usually restricted to the duration of a single sensor which has FDA

approval for only 72 hours. Our data would caution against using short duration of CGM to diagnose a patient with impaired awareness of hypoglycaemia as suggested by Kubiak et al[19].

Another possibility is that while the frequencies of low interstitial glucose (LIG) may be similar, the episodes of biochemical hypoglycaemia may differ in terms of duration or depth. In patients with IAH, with reduced or absent symptomatic or counterregulatory responses, we may expect episodes of hypoglycaemia to persist for longer before being recognised and treated and we would expect a greater proportion of time in the patients with IAH at the lower threshold of $LIG_{2.2}$ compared to $LIG_{3.0}$. However, the median duration of each episode or the total time spent with interstitial glucose either ≤ 3.0 mmol/l or ≤ 2.2 mmol/l did not differ between the groups. As the CGM system cannot record below 2.2mmol/l, it was not possible to determine what proportion of time might have been spent at very low glucose levels.

Concern has been expressed about the accuracy of CGM, particularly at hypoglycaemic levels [22] because of the physiological delay between blood and interstitial glucose which may be exaggerated when blood glucose is falling rapidly[23]. In patients with type 1 diabetes during experimental hypoglycaemia, CGM underestimates interstitial tissue glucose concentrations [24] and thus may overestimate the frequency of hypoglycaemia. Another problem when interpreting CGM traces is that if the sensor fails, the trace “flat-lines” which could be interpreted as persistent hypoglycaemia. We minimised the likelihood of this occurring by discarding any data that did not meet stringent accuracy criteria or were not obtained between two paired readings. In addition, traces were analysed by two independent observers with a third observer to adjudicate in the event of disagreement [25].

80% of episodes of symptomatic hypoglycaemia were associated with sensor glucose $<3.5\text{mmol/l}$ similar to previous reports[12]. However only 10% of episodes of LIG were associated with symptomatic hypoglycaemia and this was not different between those with IAH and NAH. This suggests low specificity of LIG to identify clinically relevant hypoglycaemia. The high proportion of LIG with no symptoms, even in those with NAH does raise concerns about the clinical relevance of these episodes and the validity of using them to diagnose IAH.

The results of the present study would suggest that, despite exposure to a similar frequency and duration of biochemical hypoglycaemia, patients with IAH remain three times more susceptible to severe hypoglycaemia, and this may indicate a difference in their ability to self-treat in the presence of profound neuroglycopenia. Some studies have found that low serum ACE concentrations may predict a lower risk of severe hypoglycaemia, despite a similar rate of biochemical hypoglycaemia [26-28] although this was not seen in other studies[29] or in our study. Patients with IAH have a reduced global brain glucose uptake during hypoglycaemia compared to those with NAH, with reduced uptake in the sub-thalamic regions of the brain, which are known to be important in the generation of physiological responses to hypoglycaemia [30]. It is unclear whether these differences could explain why people with IAH are more susceptible to SH than those with normal awareness despite similar exposure to biochemical hypoglycaemia.

In conclusion, while our study confirms the higher risks of severe (3-fold) and biochemical (1.6-fold) hypoglycaemia in patients with IAH, five days of continuous glucose monitoring is not sufficient to identify people with IAH. It also raises the possibility that patients with IAH may be less able to deal with low tissue glucose than those with normal awareness.

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Chapter 9

Prevalence of Impaired Awareness of Hypoglycaemia in Adults with Type 1 Diabetes

9.1 Introduction

In unselected populations of people with type 1 diabetes the estimated incidence of severe hypoglycaemia (requiring external help) ranges from 1.0 to > 3.0 episodes/patient/year [1-4]. Subjective recognition of the symptoms of hypoglycaemia is fundamental to effective self-management to prevent progression to severe hypoglycaemia [5, 6]. However, with increasing duration of treatment with insulin many people with type 1 diabetes experience a change in their symptoms of hypoglycaemia [7-9], manifested as either a reduction in intensity or number, or a change in symptom profile, so that neuroglycopenic symptoms predominate, while autonomic symptoms are less prominent or absent.

This diminished ability to perceive the onset of hypoglycaemia (impaired awareness of hypoglycaemia (IAH)), is alleged to affect approximately 25% of people with type 1 diabetes [10,11]. This estimate was derived from small studies conducted in the 1980s and early 1990s, which utilised a retrospective review of clinical histories. However, validated methods of assessment, which have been developed subsequently, have not been applied to a large hospital clinic-based population. In addition, as IAH is thought to be induced by recurrent exposure to hypoglycaemia, the introduction of new insulin analogues, the intensification of insulin regimens and improved methods of patient education may help to minimise exposure to hypoglycaemia and hence potentially decrease the prevalence of IAH. The present study was therefore performed to ascertain this prevalence in a randomly selected cohort of people with type 1 diabetes using the method described by Gold et al [12].

9.2 Methods

Adults with type 1 diabetes attending a diabetes outpatient clinic at the Royal Infirmary of Edinburgh (a large city teaching hospital), over a 3-year period, were recruited at random

for the survey. Inclusion criteria consisted of type 1 diabetes of more than two years duration and being aged over 16 years. Exclusion criteria were pregnancy, advanced renal failure or inability to understand or complete the questionnaire. The local medical ethics committee approved the study, and informed consent was obtained from all participants.

Each participant completed a general questionnaire to document baseline demographic characteristics and quantified the frequency of exposure to self-treated hypoglycaemia and episodes of severe hypoglycaemia (defined as requiring external assistance) during the preceding 12 months. Retrospective recall of severe hypoglycaemia over a period of one year is a robust measure in people with type 1 diabetes [13]. An investigator was present to assist with clarification of the content of the questionnaire if required.

Awareness of hypoglycaemia was assessed using the method described by Gold et al [12], which asks the question: “do you know when your hypos are commencing?” The respondent selects a number on a 7-point Likert scale with 1 representing “always aware” and 7 representing “never aware”. A score of 4 or more is designated as impaired awareness of hypoglycaemia. In addition to this subjects are asked if they have noticed a subjective alteration in their warning symptoms and their frequency of exposure to severe hypoglycaemia in the year preceding the study. Hypoglycaemia symptom scores were assessed using the Edinburgh Hypoglycaemia Scale [14].

9.2.1 Subjects

The questionnaire was completed by 518 adults with type 1 diabetes (242 male; mean (SD) HbA1c 8.4 (1.4) %; median (inter quartile range, IQR) age, 39.0 (31-50) years; duration of diabetes, 16 (9-24) years. This group were using either insulin analogues (n=384; 74%), a mixture of analogue and human insulin (n = 93; 18%) or human insulin's alone (n = 41;

8%). A basal-bolus insulin regimen was used by 82.3% (n=426), with 17.7% (n = 92) on a twice-daily regimen of fixed insulin mixtures, such as 30% soluble, 70% isophane.

9.2.2 Statistical analysis

All analyses were performed using SPSS version 12.0 for Windows. Differences between groups were analyzed using either the two-sample t test or Mann-Whitney-U test. To assess the linear relationship between two variables a Spearman correlation coefficient was calculated. A p value ≤ 0.05 was considered significant. All results are reported as mean (SD) unless otherwise stated.

9.3 Results

IAH was present in 19.5% (n = 101), (Figure 9.1). Compared to people with normal awareness of hypoglycaemia, those with IAH were significantly older ($p < 0.001$) and had diabetes for longer ($p < 0.001$) (Table 9.1). The rate of severe hypoglycaemia in the preceding year was six-fold higher in those with IAH compared to those with normal awareness ($p < 0.001$), (Figure 9.1). The prevalence of SH in the preceding year was 20.1% in the group with normal awareness and 50.5% in those with IAH (Figure 9.1). The reported intensity of autonomic symptoms was lower during episodes of self-treated hypoglycaemia in those with impaired awareness compared to those with normal awareness ($p = 0.004$). No statistical differences were observed between the groups (IAH versus normal awareness) in the intensity of neuroglycopenic symptoms ($p = 0.44$). No differences were also observed with respect to glycaemic control (HbA1c 8.3 (1.4) % vs. 8.4 (1.4) %, $p = 0.92$). A moderate and highly significant association was observed between

Figure 9.1: Prevalence and incidence of severe hypoglycaemia (SH) in the year preceding the survey of 518 adults with type 1 diabetes, with, and without, impaired awareness of hypoglycaemia.

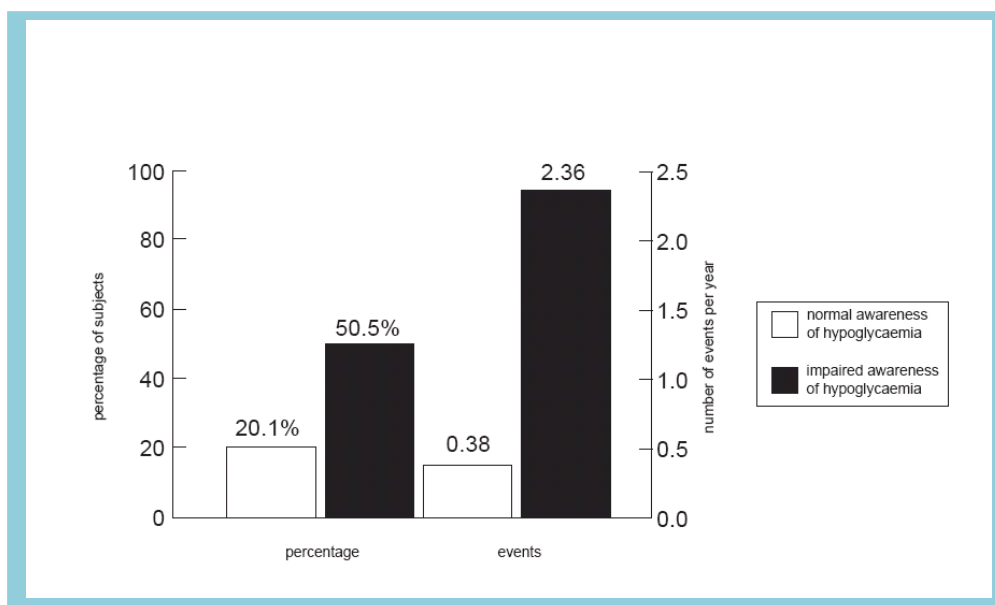


Table 9.1: Clinical characteristics of participants with type 1 diabetes by awareness of hypoglycaemia

Awareness	Normal	Impaired	P
Number (%)	417 (80.5%)	101 (19.5%)	-
Age (years)	39.3 (12.9)	45.9 (13.5)	< 0.001
Duration (years) (Median, IQR)	14 (8-22)	23 (14-32)	< 0.001
HbA1c (%)	8.3 (1.4)	8.4 (1.4)	0.92
Incidence of SH in preceding year	0.38 (1.0)	2.36 (4.8)	< 0.001
Autonomic symptoms	2.94 (1.1)	2.05 (1.2)	0.004
Neuroglycopenic symptoms	2.35 (1.0)	2.40 (1.1)	0.44
Malaise symptoms	1.95 (1.2)	2.2 (1.4)	0.56

IAH and duration of diabetes ($r_s = 0.21$, $p = < 0.001$) and between IAH and rate of SH ($r_s = 0.34$, $p = < 0.001$).

9.4 Discussion

The present survey, using a validated method of assessment, has demonstrated a prevalence of IAH of approximately 20% in an unselected adult population with type 1 diabetes. This is similar to previous estimates made 15-20 years ago that were derived on clinical history from hospital and community-based populations [9, 10, 15, 16]. Those with IAH were older, had a longer duration of diabetes, and had a six-fold higher frequency of episodes of severe hypoglycaemia and a lower intensity of autonomic symptoms during hypoglycaemia, all of which are consistent with recognised characteristics of this acquired syndrome [9, 12, and 16]. Thus the method of Gold et al appears to be sufficiently discriminating in the identification of those with IAH.

A potential limitation in defining the prevalence of IAH with precision is that this is not an “all or nothing” phenomenon. Several studies of people with [19], and without, type 1 diabetes [20-22] have suggested that exposure to antecedent hypoglycaemia can shift the glycaemic thresholds for cognitive dysfunction, symptom generation and counterregulatory hormonal secretion to lower blood glucose levels, while strict avoidance of hypoglycaemia can restore normal responses [23].

Views differ regarding the most appropriate methods and situations in which evaluation of the awareness of hypoglycaemia should be undertaken, ranging from the use of questionnaires, identification of symptom generation during experimental hypoglycaemia using glucose clamps and utilisation of continuous glucose monitoring (CGMS). In my

opinion as stated previously, IAH should be evaluated within the everyday experience of people treated with insulin, and not from observations carried out in the artificial setting of controlled hypoglycaemia in the laboratory. CGMS as demonstrated in Chapter 8 has no useful contributory role in identifying those with IAH. The other questionnaire method for assessing awareness of hypoglycaemia is that of Clarke et al [25] , which has been externally validated by a different group of investigators, who examined the relationship between the hypoglycaemia questionnaire, prospective blood glucose monitoring and glucose clamp studies to assess hypoglycaemia awareness [18]. As demonstrated in study 3 (Chapter 7) the Clarke [25] and Gold [12] methods have been proven to be sufficiently sensitive in identifying impaired awareness of hypoglycaemia and for their mutual concordance [17]. A strong correlation was demonstrated between the two methods ($r_{\text{est}} = 0.868$, $p = 0.001$) in identifying people with IAH. Those identified by both methods as having IAH were older, had a longer duration of diabetes, recorded more frequent episodes of biochemical hypoglycaemia over a four-week monitoring period and experienced more episodes of severe hypoglycaemia in the year preceding the study. Although methods that utilise questionnaires are not perfect, the two currently available methods, which are easy and quick to administer in the clinical setting, have been externally validated, and demonstrate close internal concordance.

The present study, which has applied a specific method of assessing hypoglycaemia awareness in a large outpatient clinic population (using treatments with insulin analogues and MDI), has confirmed that the prevalence of IAH has not changed significantly over the last 20 years, despite the introduction of novel therapies.

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Chapter 10

Conclusions and Future Research

10.1 Introduction

The DCCT [1] and its follow-up study the EDIC [2] have provided conclusive evidence for the need to strive for strict glycaemic control in order to minimise both microvascular and macrovascular complications. Strict glycaemic control however usually comes at a price: an increased frequency of hypoglycaemia. If hypoglycaemia simply generated some unpleasant symptoms to alert the person with diabetes to the fact that the blood glucose concentration had fallen and had no effect on cognitive function then strict glycaemic control maybe a realistic goal for many with type 1 diabetes. However, cognitive impairment does occur, which both prevents self-treatment and can cause dangerous or inappropriate behaviour. This therefore remains an important issue for people with type 1 and insulin treated type 2 diabetes and hopefully the data presented in this thesis has extended our knowledge of the effects of hypoglycaemia on cognitive function and the clinical effect of IAH.

10.2 Effects of acute insulin-induced hypoglycaemia on psychomotor function (chapter 5)

The aim of study one was to examine the effects of insulin-induced hypoglycaemia on psychomotor function in both healthy volunteers and people with type 1 diabetes. Acute hypoglycaemia caused significant impairment of several psychomotor functions in non-diabetic adults, a lower magnitude of impairment was observed in those with type 1 diabetes. The mechanism underlying this discrepant effect of hypoglycaemia on psychomotor function remains unknown. The groups differed (as would be expected) in the magnitude of sympathoadrenal activation mounted in response to the episode of insulin-induced hypoglycaemia, with the healthy volunteers producing significantly higher

concentrations of both adrenaline and noradrenaline, which potentially could interfere with performance on psychomotor testing. Symptoms however are neurally derived from activation (via centres in the hypothalamus) of the sympathetic and parasympathetic divisions of the autonomic nervous system, with the direct effect of neural stimulation of end organs (such as sweat glands) [3]. The blood glucose concentration obtained of 2.5 mmol/L during hypoglycaemia should have lead to symptom generation in both the healthy volunteers and people with diabetes who were selected to have normal awareness of hypoglycaemia and a low risk of hypoglycaemia. No significant difference, from the study described in chapter 5, in autonomic symptoms was demonstrable between the groups ($p = 0.274$).

Direct observation of the participants with and without type 1 diabetes, indicated that the participants with type 1 diabetes appeared to cope better under the condition of hypoglycaemia. They focused more on the tasks and were less distracted than the healthy volunteers. This may have arisen through previous exposure to hypoglycaemia. If mild hypoglycaemia in the person with type 1 diabetes occurs on average two times per week [4, 5] some of these episodes will occur while carrying out tasks of everyday living, thus potentially conferring a behavioural advantage over a group of hypoglycaemia naïve healthy volunteers.

Cerebral adaptation to cognitive function does occur with certain cognitive tests. As described above has the previous exposure to hypoglycaemia in the subjects with type 1 diabetes lead to cerebral adaptation. Ideally future research would examine this in a group of healthy volunteers whereby hypoglycaemia is induced recurrently with cognitive function testing and functional brain imaging carried out at each session to ascertain if cerebral adaptation does occur. This is problematical for two reasons. One, nowadays it would be deemed unethical to induce recurrent episodes of hypoglycaemia in healthy

volunteers and secondly the exposure from repeated MRI scans would be higher than the recommended amount as set out by the International Commission on Non-Ionising Radiation Protection. Until this changes and it is possible to do serial brain imaging this question may remain unsolved.

10.3 Modelling the Effects of Hypoglycaemia on a Two-Choice Task in Adult Humans

The above study demonstrated that hypoglycaemia affected central processing and not the quantity of evidence required to make a decision, or peripheral and motor processes. This study is unique in that it is the first to dissect the effect of hypoglycaemia on cognitive function this way. Previous research utilising standard psychometric tasks (which provide only a total score) only tell us that the domain to which the test relates is affected by hypoglycaemia. For example if we accept that Digit Symbol is a measure of ‘processing speed’ and state that, if Digit Symbol performance shows a decrement during hypoglycaemia, then hypoglycaemia affects ‘processing speed’. What the current task and its model allowed us to do was to dissect in more detail the processing stages that are affected. Having now demonstrated that it affects central processing the next logical step forward for further studies would be to examine whether ingestion of exogenous substances such as amino acids which have been demonstrated to preserve cognitive function during hypoglycaemia (as discussed in Chapter 3) do this by preserving central processing speeds.

10.4 An evaluation of methods of assessing impaired awareness of hypoglycaemia in Type 1 Diabetes

The main findings of this study was that the participants identified as having IAH by the methods of Gold [6] and Clarke [7] had recognised clinical characteristics of this acquired syndrome such as older age, a longer duration of diabetes etc, had a higher rate of biochemical hypoglycaemia over the 4-week monitoring period and reported a higher incidence of severe hypoglycaemia in the year preceding the study compared to those with normal awareness. The Pedersen-Bjergaard method [8] was much less discriminating and should not be used to identify those with IAH. In both the Gold and Clarke methods a score of 1 or 2 signifies normal awareness of hypoglycaemia and a score of 4 or more impaired awareness of hypoglycaemia. In this study a few people scored 3 and hence were unable to be classified as normal or impaired awareness. Further research is needed to refine these methods by studying the group that are currently unclassified to ascertain whether their clinical characteristics and frequency of hypoglycaemia can allow them to be allocated to one group. Given that both the Gold and Clarke scores are continuous variables it would be nice to ascertain if there is a positive relationship between score on these methods and incidence of hypoglycaemia.

10.5 Frequency of hypoglycaemia in adults with and without impaired awareness of hypoglycaemia

In this study intermittent home glucose monitoring data suggested a 1.6-fold greater risk of hypoglycaemia in those with impaired awareness of hypoglycaemia but there was no difference in the rate of patient- reported hypoglycaemia and five days of continuous glucose monitoring did not identify a greater frequency, duration or severity of biochemical

hypoglycaemia between NAH and IAH. Finally, the data does not support the use of brief duration of CGM to diagnose impaired awareness of hypoglycaemia. It is unknown however if a longer period of CGM would be sufficient to differentiate between those with and without this clinical syndrome. The CGM data was collected 5 to 6 years ago and significant improvements have been not only in monitor design but the interpretation of the data extracted from this. Despite this the Clarke team have reported using the Continuous Glucose Error Grid Analysis (CG-EGA) in May 2010 [9], that compared to the Yellow Springs blood glucose analyser (during hypoglycaemic conditions) the “clinically accurate”, “benign errors” and “clinical errors” for home blood glucose monitoring were 83.5%, 6.4% and 10.1% and for CGM 57.1%, 8.4% and 34.5% respectively. Until CGM can accurately identify and not over report the frequency of hypoglycaemia there is little to be gained by repeating this study till then. Therefore in conclusion at the present time there is no role for CGM in diagnosing individuals with IAH.

10.6 Prevalence of Impaired Awareness of Hypoglycaemia in Adults with Type 1 Diabetes

The main finding in this study is that despite the recent improvements in insulin manufacture, diabetes technology and diabetes education the prevalence of IAH has remained relatively static at nearly 20%. The strength of the study was that a large number of subjects participated (n=518), representing nearly a third of all people with Type 1 diabetes who attended clinic at a large University teaching hospital. The group however was heterogeneous and if the idea was to ascertain the effect of recent improvements then only people on these such as insulin analogues or basal bolus injections/insulin pumps should

have been examined. In reality although they contributed the vast majority of participants with 74% on analogues and 82.3% on basal bolus participants on human insulin given twice a day were included. Further research should examine only those that have been on analogues and intensive insulin regimes since at or shortly after diagnosis to examine these effects of the prevalence of IAH which you would expected should be lower due to the lower reported rates on hypoglycaemia on these regimens.

10.7 References

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Appendix 1

Published papers

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